COMMISSION OF THE EUROPEAN COMMUNITIES

EURATOM

RADIATION PROTECTION

PROGRAMME

PROGRESS REPORT

1988

EUR 12064 DE/EN/FR

COMISIÓN DE LAS COMUNIDADES EUROPEAS
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KOMMISSION DER EUROPAISCHEN GEMEINSCHAFTEN
ΕΠΙΤΡΟΠΗ ΤΩΝ ΕΥΡΩΠΑΪΚΩΝ ΚΟΙΝΟΤΗΤΩΝ
COMMISSION OF THE EUROPEAN COMMUNITIES
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EURATOM

Relación de actividades
Programa

PROTECCIÓN RADIOLÓGICA

Beretning
Program

STRÅLINGSBESKYTTELSE

Tätigkeitsbericht
Programm

STRAHLENSCHUTZ

Έκθεση πεπραγμένων
Πρόγραμμα

ΠΡΟΣΤΑΣΙΑ ΑΠΟ ΑΚΤΙΝΟΒΟΛΙΕΣ

Progress report

RADIATION PROTECTION

programme

Rapport d'activité
Programme

RADIOPROTECTION

Rapporto d'attività
Programma

RADIOPROTEZIONE

Verslag van de werkzaamheden
Programma

STRALINGSBESCHERMING

Relatório de actividades
Programa

RADIOPROTECÇÃO

1988

1989 EUR 12064 DE/EN/FR

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TABLE OF CONTENTS

TABLE DES MATIERES

* Schlussbericht / Final report / Rapport final

** Bericht noch nicht verfügbar / Report not yet available / Rapport pas encore disponible

I

EINLEITUNG

INTRODUCTION

INTRODUCTION

Vorwort

Der Tätigkeitsbericht 1988 des Strahlenschutzprogramms der Europäischen Gemeinschaften fasst die neuesten Ergebnisse aus den ca. 320 Forschungsvorhaben zusammen, die 1988 etwa 700 wissenschaftliche Veröffentlichungen ergeben haben. Weiterhin, treffen jetzt die Ergebnisse von den Post-Tschernobylaktionen ein, die nach der am 21. Dezember 1987 durch den Ministerrat angenommen Revision des Strahlenschutzprogramms eingeleitet wurden. Die ersten Ergebnisse dieser Untersuchungen werden später veröffentlicht werden.

Der vorliegende Bericht illustriert die weite Spanne von Problemen, die in der Strahlenschutzforschung berücksichtigt werden müssen, damit sie die wissenschaftliche Grundlage für den Schutz des Menschen und seiner Umgebung von möglichen schädlichen Folgen ionisierender Strahlen zur Verfügung stellen kann. Viele der Aspekte, die jetzt im Programm betont werden, waren vor 10 Jahren fast unbekannt, wie beispielsweise die Probleme der Radonexposition in Innenräumen, die Optimierung der medizinisch-diagnostischen Radiologie, die Abschätzung der Folgen von Strahlenunfällen und deren Bewältigung, die Optimierung des Strahlenschutzes von Arbeitern und die Wirkungen niedriger Strahlendosen. Die sorgfältige Durchsicht der wissenschaftlichen Berichte und der hier angeführten Workshops zeigt deutlich das Aussmass, in welchem diese Aspekte in die gegenwärtige Forschung auf diesem Gebiet einbezogen sind.

Die Forschung im Strahlenschutz verlangt eine Annäherung vieler verschiedener Fachrichtungen und den Austausch von Informationen und Meinungen zwischen den Forschern, deren Interesse von der Grundlagenforschung über ordnungspolitische Tätigkeiten bis zur praktischen Anwendung reicht. Die Förderung des Informationsaustausches und der Zusammenarbeit zwischen Wissenschaftlern in der Gemeinschaft ist deshalb ein Hauptanliegen des Programms. Dies geht auch hervor aus den 18 Treffen von Studiengruppen mit Vertragsnehmern und eingeladenen Sachverständigen und den 36 Workshops mit internationaler Beiteiligung, die 1988 organisiert wurden, wie auch aus den 15 veröffentlichten Sitzungsberichten. Diese Aktivitäten stellen zusammen mit den wissenschaftlichen Veröffentlichungen aus der Vertragsforschung einen namhaften Beitrag zu den weltweiten Forschungsaktivitäten im Strahlenschutz dar. Durch den direkten Einfluss des Programms und der Projektgruppen, die es gebildet hat und fördert, ist die Zusammenarbeit zwischen den Wissenschaftlern, die in der Strahlenschutzforschung tätig sind, sehr intensiv und wirksam geworden. Dies hat zur Schaffung eines "Europas für die Wissenschaftler" beigetragen, das ein wichtiger Faktor für die Zukunft der Europäischen Gemeinschaften darstellt. Das Programm hat ausserdem die Absichtserklärungen mit den USA und Kanada weiter entwickelt und hat seine Beziehungen zu anderen Ländern ausserhalb der Gemeinschaft ausgedehnt, um gemeinsam Prioritäten festzulegen und Verdopplung der Forschung zu vermeiden.

Viele Fragen im Strahlenschutz müssen noch durch Forschung gelöst werden und diese Notwendigkeit zeigt sich auch in der weiter ansteigenden Beunruhigung der Bevölkerung über Strahlenrisiken. Ein Vorschlag für die Fortführung des Programms wurde entworfen und dem Ministerrat zur Entscheidung unterbreitet. Der neue Vorschlag ist nicht einfach nur eine Fortsetzung der gegenwärtigen Forschung, sondern bemüht sich um einen umfassenderen Ansatz, in dem a) die Art und Weise, auf die der Mensch einer Strahlenexposition ausgesetzt ist und das Aussmass dieser Exposition zu bestimmen, b) die Folgen einer solchen Exposition abzuschätzen und nach Mitteln zu suchen, sie zu verhüten oder zu behandeln, c) die Risiken der Bestrahlung zu bewerten und den Strahlenschutz zu optimieren. Zukünftig wird das Programm verstärkt seine Aufmerksamkeit auch auf ein anderes wichtiges Problem lenken, den Mangel an jungen gutausgebildeten Wissenschaftlern, die sich mit dem Strahlenschutz befassen wollen.

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Foreword

The 1988 Progress Report of the Radiation Protection Programme of the Commission of the European Communities summarizes the most recent results coming from some 320 research projects which have led to some 700 scientific publications in 1988. In addition, results are now coming from the post-Chernobyl actions initiated following the revision of the Radiation Protection Programme adopted by the Council on 21 December 1987. The first results of these studies will be published later.

The present report illustrates the wide range of topics that radiation protection research has to consider in order to provide the scientific background for the protection of man and his environment from the possible harmful effects of ionizing radiation. Many of the aspects now given emphasis in the Programme were barely heard of a decade ago, such as the problems related to indoor radon exposure, optimisation of medical diagnostic radiology, assessment of radiation accidents consequences and their management, optimisation of the radiation protection of workers and the effects of very low dose exposure. A perusal of the scientific reports and the meetings listed in this report will readily show the extent to which these aspects are involved in current research in this area.

Research in radiation protection demands an approach involving many different disciplines and requires an exchange of information and opinion between scientists whose interests range from basic science to regulatory activities and practical applications. Promotion of the exchange of information and cooperation between scientists in the Community is therefore of prime importance for the Programme. This is demonstrated by the 18 study group meetings with contractors and invited experts and 36 workshops with international participation organized in 1988, and the 15 published proceedings. These activities, together with the publications originating from contract work, represent a substantial proportion of the worldwide research efforts in radiation protection. Cooperation between scientists working in radiation protection research has become very close and effective as a result of the direct efforts of the Programme and of the cooperative groups of scientists initiated and supported by the Programme. This has contributed to the creation of an "Europe for Research Workers" which will be an important factor for the future of the European Communities. Moreover, the Programme has continued to implement the Memoranda of Understanding with the USA and Canada and has extended its contacts with other countries outside the Community to define priorities and avoid duplication of research.

Many questions in radiation protection remain to be solved by research, the need for which is underlined by the public concern about radiation risks which continues to escalate. A proposal for a continuation of the Programme has been elaborated and submitted to the Council of Ministers for decision. The new proposal does not simply continue the present lines of research but rather integrates them into a more comprehensive approach a) to determine the ways by which man is exposed to radiation and the extent of such exposure, b) to evaluate the consequences of such exposure and to search for means to prevent and treat them, and c) to assess the risks of radiation and optimise radiation protection practices. In the future, the Programme will also address itself to another important problem which concerns the shortage of young, well-trained scientists entering the field of radiation protection.

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Nuclear Safety, Waste Management,
Prevention and Control of Pollution

Preface

Le présent rapport d'activité 1988 du Programme Radioprotection de la Commission des Communautés européennes résume les résultats les plus récents de quelque 320 projets de recherches ayant abouti à la publication de quelque 700 articles scientifiques en 1988. De plus, les actions post-Tchernobyl entamées suite à la révision du Programme Radioprotection adoptée par le Conseil le 21 décembre 1987, donnent leurs premiers résultats. Ceux-ci seront publiés plus tard.

Le présent rapport montre combien est étendu le domaine que la recherche en radioprotection doit considérer pour fournir les bases scientifiques nécessaires à la protection de l'homme et de son environnement contre les effets nocifs éventuels des rayonnements ionisants. Beaucoup d'aspects sur lesquels le programme met actuellement l'accent étaient peu pris en considération il y a une décade, citons les problèmes liés à l'exposition au radon à l'intérieur des bâtiments, l'optimisation du diagnostic radiologique, l'estimation des conséquences d'un accident radiologique et leur gestion, l'optimisation de la protection radiologique des travailleurs et, enfin, les effets de l'exposition à très faible dose. Un examen des rapports scientifiques et des réunions repris dans ce rapport montre à quel point ces nouveaux aspects sont développés dans la recherche actuelle.

La recherche en radioprotection exige une approche impliquant de nombreuses disciplines différentes et un échange d'information et d'opinion entre des scientifiques dont le centre d'intérêt est la recherche fondamentale, la réglementation ou les applications pratiques. La stimulation de l'échange d'information et la coopération entre les scientifiques dans la Communauté sont, par conséquent de prime importance pour le programme. Ceci est démontré par les 18 réunions de contractants et d'experts invités, par les 36 ateliers à participations internationales organisés en 1988 et par les 15 actes publiés. Ces activités, de même que les publications découlant des contrats de recherche, représentent une part substantielle des efforts de recherche mondiale dans le domaine de la radioprotection. La coopération entre les scientifiques travaillant dans la recherche en radioprotection est devenue très étroite et très efficace grâce aux efforts directs du programme et des groupes scientifiques mis sur pied et soutenus par le programme. Ceci a contribué à la création d'une "Europe des chercheurs" qui sera un facteur important pour le futur des Communautés européennes. En outre, le programme a poursuivi la mise en oeuvre de la déclaration d'intention avec les Etats Unis d'Amérique et le Canada et a étendu ses contacts avec d'autres pays non communautaires afin de définir des priorités et d'éviter la duplication de recherche.

Beaucoup de questions en radioprotection restent encore à résoudre par la recherche, et le besoin s'en fait sentir par la préoccupation croissante du public en ce qui concerne les risques dûs aux rayonnements. Une proposition pour une continuation du programme a été élaborée et soumise au Conseil des ministres. La nouvelle proposition n'est pas qu'une simple poursuite des lignes de recherche actuelles mais les intègre plutôt dans une approche plus globale: a) détermination des voies par lesquelles l'homme est exposé aux rayonnements et l'importance de cette exposition, b) évaluation des conséquences d'une telle exposition et des moyens de prévention et de traitement et c) détermination des risques liés à l'irradiation et optimisation des pratiques de radioprotection. Dans le futur, le programme abordera également un autre problème important, celui du manque de formation, dans le domaine de la radioprotection, de jeunes scientifiques.

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G.B. Gerber
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Radioprotection

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Sécurité nucléaire, impact de
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et gestion des déchets

II

Mitglieder und Experten 1988

Beratender Verwaltungs- und Koordinierungsausschuss "STRAHLENSCHUTZ"

Members and experts 1988

Management and Coordination Advisory Committee "RADIATION PROTECTION"

Membres et experts 1988

Comité consultatif en matière de Gestion et de Coordination "RADIOPROTECTION"

Mitglieder und Experten 1988
Beratender Verwaltungs- und Koordinierungsausschuss "STRAHLENSCHUTZ"

Members and experts 1988
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III

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHE EN RADIOPROTECTION

III A

STRAHLENDOSIMETRIE UND IHRE INTERPRETATION

RADIATION DOSIMETRY AND ITS INTERPRETATION

DOSIMETRIE DES RAYONNEMENTS ET SON INTERPRETATION

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Association pour le Développement
de la Physique Atomique, ADPA
118, route de Narbonne
F-31062 Toulouse Cedex

Contract no.: BI6-A-292-F

Head(s) of research team(s) [name(s) and address(es)]:

Prof. D. Blanc
Centre de Physique Atomique
Université Paul Sabatier
118, route de Narbonne
F-31062 Toulouse Cedex

Telephone number: 61-556857

Title of the research contract:

Development of a general method allowing the complete modellisation of proportional counters.

List of projects:

1. Development of a general method allowing the complete modellisation of proportional counters.

Title of the project n^0. : B16-A-292-F

Development of a general method allowing the complete modellisation of proportional counters

Heads of project :

P.Ségur, Directeur de Recherche au CNRS
J.P Boeuf, Chargé de Recherche au C.N.R.S.

Scientific staff :

I. Pérès, Allocataire du M.R.E.S.
J.P Boeuf, Chargé de Recherche au CNRS
M.C Bordage, Chargée de Recherche au CNRS
P.Ségur, Directeur de Recherche au CNRS

I. Objectives of the project :

Our purpose is to carry out a systematic modelling of the motion of ions and electrons in a proportional counter. This modelling will be useful in order to improve our knowledge of the working properties of cylindrical proportional counters. This best knowledge will allow us to determine the optima geometrical, electrical and physical parameters in order to achieve a miniaturization of the counters specialy needed for microdosimetric purpose (Rossi type counters for example). In this case, the determination of the optimum gain is very important and the best way in order to predict the gain variation, due to change in geometrical or electrical parameters, is the calculation of the various transport parameters.

II. Objectives for Reporting period :

Transport parameters which characterize the electron motion in the counter (drift velocity, diffusion coefficients, ionization coefficients) will be calculated. These calculations will be made in organic molecular gases and in their mixtures, which are used in the field of microdosimetry and for which a few number of data (electron molecule cross sections for example) are at the moment available. The determination of these transport parameters is necessary to achieve the second part of this program concerning the macroscopic modelling of the discharge evolution in the counter.

1-Methodology

The calculation of swarm parameters in a cylindrical proportional counter is made difficult because, since the electric field increases very quickly near the anode wire, these swarm parameters are expected to depend very strongly on the position. Then a very complicated numerical solution of the Boltzmann equation must be made. At the moment no solution of this type can be found in the literature. In order to determine the variation as a function of space of the swarm parameters (specially the ionization coefficient), people generally use some approximate analytical expression, depending on some arbitrary parameters, and they determine these parameters by fitting calculated and measured values of the gas gain. It is obvious that this procedure is strongly arbitrary and, in any case, needs the knowledge of experimental values of the gas gain in order to predict the behaviour of ionization coefficient.

The determination of the ionization coefficient and of the gas gain without the help of experimental determination, requires the Boltzmann equation resolution. The only data needed to solve this equation are the various electron-molecule cross sections for the different gases. When these cross sections have been obtained, all the swarm parameters may be calculated for the gases studied and also for their mixtures. Furthermore, it is very difficult (and very time consuming) to solve the Boltzmann equation in a general way, so it is interesting to develop (together with the general solution for a cylindrical geometry) some simplified solution or a solution for a simplified Boltzmann equation.

The most popular simplification is to assume that the swarm parameters depend on the ratio E/P, where E is the electric field and P the pressure and that their space variation is known through the space variation of the electric field. To calculate these quantities, it is then only necessary to solve the Boltzmann equation where the gradients with respect to space have been supressed. The swarm parameters obtained in this case are called 'equilibrium swarm parameters' since suppressing space gradients is equivalent to assume that electrons are in equilibrium with the electric field (i.e. mean energy gain due to electric field is exactly balanced by mean energy losses due to collisions). It must be emphasized now that from the usual experimental determination of swarm parameters only equilibrium values can be obtained. When these experimental values are used to calculate the gas gain, it may be asked if the values obtained can be compared with experimental determination. Then the question is : are the real (non equilibrium) values of swarm parameters different or not from the corresponding equilibrium values? To answer this question, it is necessary to solve the Boltzmann equation in equilibrium and non equilibrium situations respectively. This is what we have made during the first year of this work.

Our work has been divided into three parts :

a) Determination of electron molecule cross sections for a lot of various gases;

b) Numerical solution of the Boltzmann equation and determination of swarm parameters in equilibrium situations;

c) Numerical solution of the Boltzmann equation in non equilibrium situations by using the Monte carlo method.

2- Results

The key for our calculations is the determination of the various electron molecule cross sections. In the case of organic molecular gases, the knowledge of these cross sections is very bad and, in some cases (for the most complex molecules) no cross section is available. This is the reason why we are carrying out a systematic investigation of these cross sections for a great number of atomic and molecular gases. This determination must be made for a large electron energy range (from zero to 10^6 electron volts) because, due to the very important values of the electric field in the vicinity of the anodic wire of the counter, electrons may have very high energies. We have made these determinations for argon, nitrogen, oxygen, carbon dioxyde and methane.

The equilibrium swarm parameters have been determined by solving the equilibrium form of the Boltzmann equation and the calculations have been made for all the gases given above. Some calculations have also been made in tissue equivalent mixtures (CH_4-N_2-CO_2 mixtures). The swarm parameters calculated in these tissue equivalent mixtures are the first, to our knowledge, available in the litterature.

The results obtained in the non equilibrium case show that the real (non equilibrium) ionization coefficient depend on the pressure-distance product (P r) and also on the parameter K = V/log(b/a) (V being the voltage applied and b (resp. a) the radius of the cathode (resp. the anode)). Furthermore the real values of the ionization coefficient α are (at intermediate E/P values) lower than equilibrium values and, on the contrary, for high E/P (which is the case for very low pressures or very short anode radius), the real ionization coefficient becomes higher than equilibrium results. These differences between equilibrium and non equilibrium results, can be easily explained. For intermediate E/P, as E is quickly increasing, electrons undergo many collisions in low field regions and they cannot follow the fast variation of the electric field : they are delayed; their mean energy is lower than the equilibrium mean energy and consequently their ionization coefficient is decreased. For high electric fields, near the anodic wire, electrons can turn around the wire and, at low pressures or for very short anode radius (when the electron mean free path is higher than the anode radius), the electron trajectory is longer and then there is an increase in the ionization coefficient.

Comparisons which can be made between equilibrium calculated and measured values of the gas gain, must take into account the above results. The calculated gain with the use of equilibrium values of α must be in any case different of the measured values. Differences are very low if the gas does not strongly slow down the electrons (atomic gases); on the contrary, differences are very large if the gas strongly slows down the electrons (molecular gases).

3-Discussion

In our opinion, the objective planned for this reporting period has been fulled reached since we have not only made equilibrium calculations of the ionization coefficient (and of all the swarm parameters), but we have also shown the importance of non equilibrium effects on the electron motion in this type of geometry.

IV. Objectives for the next reporting period :

The second part of this program will be devoted to the macroscopic modelling (based upon the simultaneous resolution of the continuity equations for electrons and ions coupled to the Poisson equation) of the motion of electrons and ions in a cylindrical proportional counter. This study will lead to a determination of the time variation of the voltage pulse generated by the motion of charged particles in the counter. This result will firstly allow the 'real' gain of the counter corresponding to the ratio of the pulse heights obtained in proportional and ionization chamber modes to be calculated. It will then be possible to determine the end of the proportionality regime (due to the generation of the positive ion space charge) and to define the extreme conditions of the working properties of the counter.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]

J. Barthe and G. Portal,
C.E.N. de Fontenay aux Roses,
D.P.T.-S.I.D.R.
BP n° 6
92260 Fontenay aux Roses, France.

VI. Publications :

P. Ségur, I. Pérès, J.P. Boeuf and M.C. Bordage,
Microscopic calculation of the gas gain in cylindrical proportional counters,
accepted for publication in Radiation Protection and Dosimetry.

I. Pérès, P. Ségur and J.P. Boeuf,
Numerical Modelling of Space Charge effects in Cylindrical Proportional Counter, submitted to the XIXth International Conference on Phenomena in Ionized Gases, Belgrade, July 1989.

M.C. Bordage, P. Ségur and I. Pérès,
Calculation of Ionization Coefficient at High E/N Values in Helium, Argon and Methane,
submitted to the XIXth International Conference on Phenomena in Ionized Gases, Belgrade, July 1989.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Association pour le Développement
de la Physique Atomique, ADPA
118, route de Narbonne
F-31062 Toulouse Cédex

Contract no.: BI6-A-001-F

Head(s) of research team(s) [name(s) and address(es)]:

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Dr. M. Terrissol
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Université Paul Sabatier
118, route de Narbonne
F-31062 Toulouse Cédex

Telephone number: 61-53.08.18

Title of the research contract:

Theoretical support to calibration of neutron area monitors in radiation protection.

List of projects:

1. Theoretical support to calibration of neutron area monitors in radiation protection.

Title of the project no.:

Determination of photon and neutron fluence spectra around the Cadarache accelerator.

Head(s) of project:

Mrs C. CAZES-FRAGNAC and M. TERRISSOL

Scientific staff:

Mrs CAZES-FRAGNAC, MM. O. BAUDEL, D. BLANC

I. Objectives of the project:

L'ensemble du travail a pour but d'apporter un support théorique aux travaux conduits par le CEA dans le domaine de la radioprotection en ambiance neutronique. La finalité étant la réalisation d'un programme de simulation sur ordinateur du transport des neutrons et des photons reproduisant fidèlement les conditions réelles et permettant de faire varier de nombreux paramètres.

II. Objectives for the reporting period:

Adaptation à la configuration géométrique du site de Cadarache. Comparaison code Monte-Carlo, mesures autour de l'accélérateur.

III. Progress achieved:

1 - Introduction

Dans le cadre de l'étalonnage des appareils de radioprotection, l'accélérateur du CEA Cadarache délivre des neutrons de 14 MeV par la réaction $d + T \rightarrow \alpha + n$. Ces neutrons sont filtrés par différents boucliers de taille et de nature variables. Le but de ce travail est d'établir un code de simulation permettant de calculer les flux et les spectres énergétiques des particules pénétrant dans un appareil de mesure, en suivant les neutrons jusqu'à 0,01 eV de façon à considérer les réactions de capture radiative. Le code doit respecter le plus possible les conditions expérimentales.

2 - Géométrie du système

Le schéma présente le système expérimental tel qu'il a été modélisé pour la simulation.

La source est supposée ponctuelle et placée au centre d'une demi-sphère d'air de 5 cm de rayon.

La demi-calotte sphérique d'uranium centrée sur la source a une épaisseur de 12 cm, la demi-calotte sphérique de fer qui la recouvre a une épaisseur de 15 cm.

En arrière de la source, un cylindre d'uranium prolonge la calotte sur 12 cm; il est entièrement recouvert d'une épaisseur de 4 cm de fer.

Le chariot de fer qui sert de guide au dispositif est représenté par un parallélépipède de 40x40x5 cm.

Le milieu est uniquement limité par le sol en béton situé à 3,30 m. La composition du sol en pourcentages massiques est :

O : 50 % , Si : 31,6 % , Fe : 10 % , Ca : 8,4 %

Le diamètre du modérateur peut varier de 10 à 30 cm et sa distance avec la source peut aller jusqu'à 1,50 m.

Tout le dispositif est situé dans l'air, dans des conditions normales de température et de pression. Nous avons pris :

N_2 : 77,8 % , O_2 : 22,2 %.

Les murs et le toit, en alliage d'aluminium, sont situés à des distances très grandes par rapport au montage et n'ont pas été pris en considération.

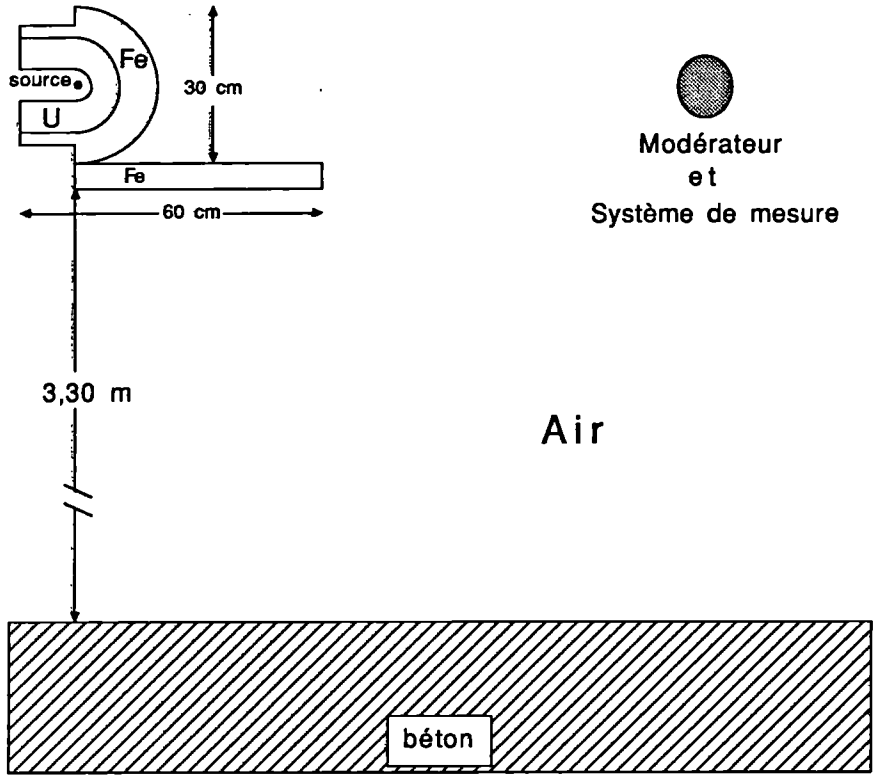


Schéma du site expérimental de Cadarache

La relative complexité des conditions géométriques et le nombre de milieux différents montrent l'intérêt du soin à apporter à la mise en forme de la bibliothèque de sections efficaces et à la simulation elle-même pour réduire la variance au niveau du modérateur.

3 - Bibliothèque de données

La bibliothèque de données provient de la banque de données de l'A.E.N. Cette bibliothèque a été générée par un système modulaire qui donne les tables de sections efficaces neutrons-gammas couplées par groupe d'énergie; les sections efficaces de base étant celles des librairies ENDF/B du Laboratoire National d' Oak Ridge.

Les sections efficaces sont moyennées dans chaque groupe i par:

$$\sigma_i = \frac{\int_i \sigma(E)W(E)dE}{\int_i W(E)dE}$$

où $\sigma(E)$ représente la section efficace ponctuelle pour l'énergie E et W(E) une fonction de pondération. Cette fonction est 1/E lorsque l'énergie des neutrons est supérieure à 0,345 eV et a une forme maxwellienne pour les énergies inférieures :

$$W(E) = C.E.\exp(-E/kT)$$

pour les photons la fonction de pondération est W=1.

La bibliothèque permet de traiter les neutrons de 15 MeV à 10-4 eV en 100 groupes d'énergies; pour les photons 21 groupes de 14 MeV à 10 keV.

4 - Réductions de variance

La géométrie du système entraîne un très faible comptage dans le modérateur et pour obtenir une précision acceptable avec des temps de calcul raisonnables nous avons été conduits à utiliser des techniques de réduction de variance. En réalité certaines techniques réduisent la variance et d'autres le temps calcul de façon à rendre minimal le produit $\sigma^2 t$, où t est le temps de calcul ayant donné σ^2 pour variance. Nous présentons uniquement ici les principales techniques qui ont été développées.

4-1 - La transformation exponentielle

Elle permet à la particule de se déplacer dans une direction préférentielle, appelée encore direction d'importance, en réduisant artificiellement la section efficace macroscopique dans une direction et en l'augmentant dans la direction opposé selon :

$$\Sigma_{ex} = \Sigma_t (1-p\mu)$$

où Σ_{ex} est la section efficace fictive, Σ_t la section efficace vraie, μ le cosinus de l'angle entre la direction préférentielle et la vitesse de la particule et p le paramètre de la transformation utilisé pour faire varier le biaisage. La direction préférentielle choisie étant celle allant de la particule au centre du détecteur (modérateur); dans l'uranium et le fer p = 0,6 , dans le béton p = 0,9 et p = 0,55 dans l'air. Par le biais de cette transformation, le poids statistique des particules diminue de façon exponentielle quand la pénétration dans la direction d'importance

augmente. On observe alors une dispersion des poids, conduisant peu à peu à une augmentation de la variance.

4-2 - Splitting et roulette russe

Pour pallier cet inconvénient nous avons introduit une fenêtre de poids permettant d'éliminer les trajectoires extérieures à l'aide du splitting et de la roulette russe : les particules qui se dirigent dans la direction d'importance sont augmentées en nombre pour fournir un meilleur échantillon et lorsqu'elles migrent dans la direction opposée elles sont éliminées de façon non biaisée. Ceci permet de garder la population à peu près constante dans la direction d'importance et dans la zone de mesure et égale pratiquement au nombre de particules source.

4-3 - Biaisage de collision isotrope

La diffusion des neutrons est très souvent isotrope; dans ce cas, on biaise la collision par l'échantillonnage de la distribution du cosinus μ de l'angle de diffusion suivant la densité de probabilité:

$$f(\mu) = \frac{p}{(1-p\mu)\mathrm{Log}(\frac{1+p}{1-p})}$$

ceci permet d'éviter une croissance continue des poids statistiques des particules.

4-4 - Splitting à partir de la source

Pour obtenir une meilleure efficacité de ces techniques, il faut les appliquer dès l'émission des particules c'est à dire sur chaque neutron source : chacun est décomposé en 100 neutrons de poids 0,01. Dans les boucliers d'uranium et de fer, nous prenons une fenêtre de poids fixe. Mais dans l'air elle varie avec la position de la particule.

5 - Simulation

Les programmes de calcul utilisent la méthode de Monte-Carlo et effectuent la simulation par groupes de particules source. A la fin de chaque groupe, un estimateur du résultat E_i est évalué ainsi que sa variance σ^2_i. Si n groupes de particules ont été simulés, on montre que l'estimateur final a une variance minimale en prenant :

$$E_{final} = \frac{1}{\sum_{i=1}^{n} \frac{1}{\sigma^2_i}} \sum_{i=1}^{n} \frac{E_i}{\sigma^2_i}$$

6 - Résultats

Le principal résultat est le spectre énergétique des neutrons pénétrant le modérateur. Afin de comparer avec les travaux expérimentaux nous calculons la fluence particulaire Φ_i en neutrons dans le détecteur pour chaque tranche d'énergie et par neutron source. A Cadarache les mesures des spectres de neutrons sont effectuées à l'aide de 9 sphères de polyéthylène type Bonner avec un détecteur central à l'hélium 3. Les spectres de protons de recul sont mesurés avec deux compteurs proportionnels à remplissage gazeux, dépendant de l'énergie et un scintillateur liquide NE213. Nous pouvons voir sur la figure, la comparaison des résultats expérimentaux et du code de calcul. En ordonnées est porté $E.\Phi(E)/\Delta E$ où $\Phi(E)$ est la fluence particulaire dans le modérateur par neutron source d'énergie moyenne E dans une tranche d'énergie de largeur ΔE. Le spectre expérimental est toujours supérieur au spectre obtenu par le calcul, mais l'allure et les pics sont conservés.Les fluctuations observées sont dues au fait que peu de particules de faible énergie atteignent le modérateur.

Les expérimentateurs mesurent aussi le Kerma dans le modérateur. Il est possible à partir des spectres obtenus par le code de calculer :

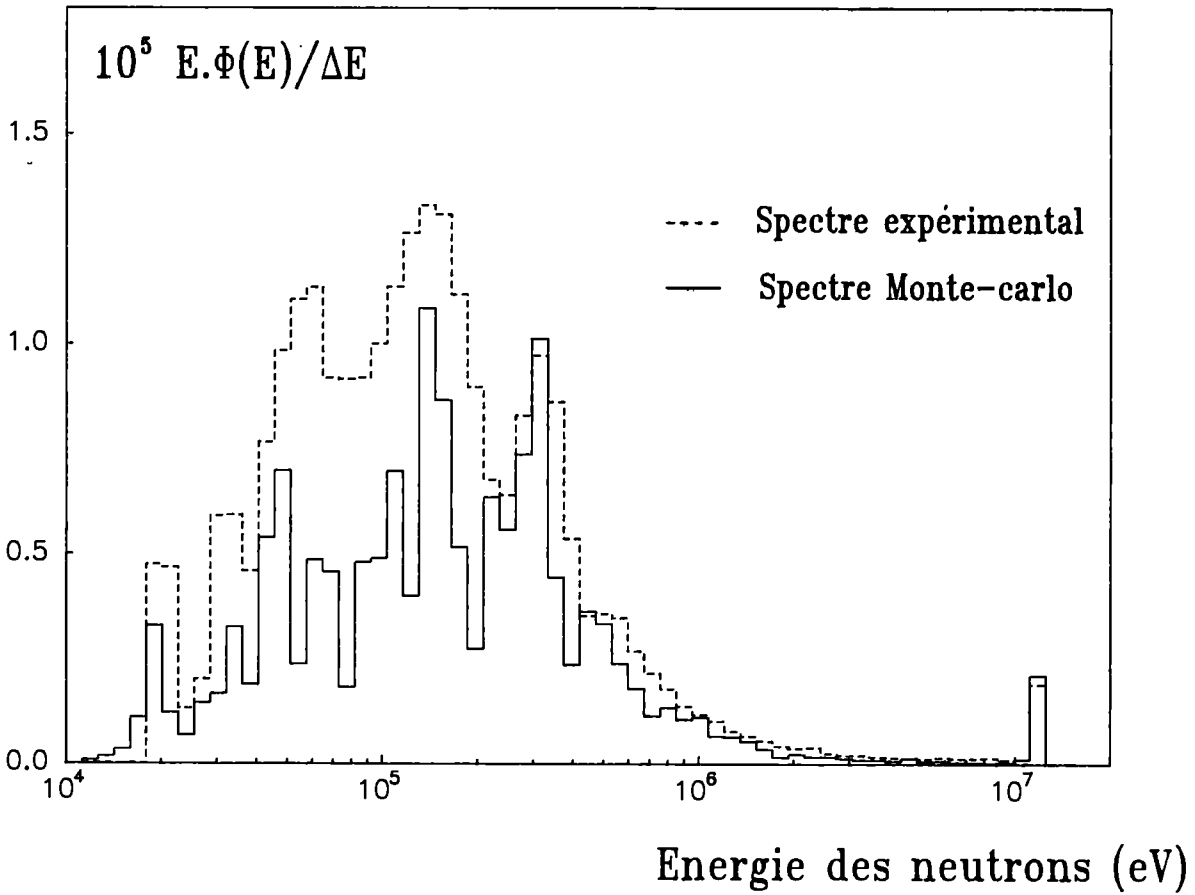
$$K = \sum_{i=1}^{n} \Phi_i k_i$$

où n est le nombre de tranches d'énergie prises en considération et k_i, le facteur de conversion fluence-Kerma. Le Kerma trouvé est :

20.10^{-15} Gray/neutron par le code Monte-Carlo
27.10^{-15} Gray/neutron par mesure expérimentale.

Pour les mesures de Kerma la technique utilise une chambre d'ionisation tissu-équivalent couplée à un compteur Geiger-Muller.

Les écarts observés ont certainement deux causes : la bibliothèque et la modèlisation. Compte tenu des positions relatives source-détecteur, la fluence mesurée dépend fortement de la forme des sections efficaces différentielles de diffusion. La bibliothèque que nous utilisons actuellement fournit les matrices qui permettent de décrire l'anisotropie des processus de


10^5 E.Φ(E)/ΔE
1.5
1.0
0.5
0.0
10^4
10^5
10^6
10^7
Spectre expérimental
Spectre Monte-carlo
Energie des neutrons (eV)

transfert de groupe à groupe par une expansion de Legendre d'ordre 8. Cette description est insuffisante pour les transferts à l'intérieur d'un même groupe d'énergie. Il faudrait obtenir d'avantage de précision pour la décomposition de la distribution de $f(\mu)$ en expansion de Legendre et pousser le développement au moins jusqu'à l'ordre 16; mais pour 100 groupes d'énergie cela demande sûrement un très gros travail, à condition toutefois de connaître les sections efficaces.

Pour améliorer la comparaison des mesures avec le code de calcul il faudrait modéliser le système de mesure lui-même afin de rendre homogène la modèlisation. La simulation d'une chambre d'ionisation ou d'un compteur proportionnel, si elle est réalisable, aiderait à la compréhension de l'écart observé. Mais l'expérimentation nécessite l'emploi de plusieurs compteurs de type et de sensibilité différents et l'introduction dans le code de calcul est pour le moment difficilement envisageable.

7 - Conclusion

Le code de calcul qui a été mis au point permet d'évaluer les spectres énergétiques des neutrons et des photons au niveau d'un détecteur dans la configuration géométrique correspondant au dispositif expérimental de Cadarache. Vu la complexité de cette dernière, la précision statistique dans certaines tranches d'énergie n'est pas parfaite. Cependant ce code donne une estimation très correcte de la fluence attendue dans chaque tranche d'énergie et va permettre l'étalonnage de différents appareils de radioprotection en ambiance neutronique. Ce travail nous a montré que la simulation précise d'un site expérimental diminuait la souplesse et l'efficacité d'un code de calcul. Lorsqu'une nouvelle bibliothèque de sections efficaces sera disponible, il sera relativement aisé de l'introduire dans la suite de programmes. Pour parfaire les comparaisons code-expérience, il faudrait sans doute simuler ce qui se passe dans le système de mesure.

IV. Objectives for the next reporting period:

Adaptation complète de la simulation aux conditions expérimentales en vue de mettre au point l'étalonnage d'appareils de radioprotection.

Comparaison simulation-mesures.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Service d'Instrumentation et de Dosimétrie en Radioprotection
Dr G. PORTAL
CEA/CEN/FAR 92265 FONTENAY AUX ROSES

Dr BUXEROLLE
CEA/DPT
Centre d'Etudes de Cadarache, B.P. N° 1
13115 SAINT PAUL LES DURANCE

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-A-180-F

Centre de Physique Atomique
Université Paul Sabatier
118, route de Narbonne
F-31062 Toulouse Cédex

Head(s) of research team(s) [name(s) and address(es)]:

Prof. D. Blanc
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F-31062 Toulouse Cédex

Dr. M. Terrissol
Centre de Physique Atomique
Université Paul Sabatier
118, route de Narbonne
F-31062 Toulouse Cédex

Telephone number:

Title of the research contract:

Simulation of low-energy electron transport as a function of time. Application to microdosimetry and radiobiology.

List of projects:

1. Electron transport calculations, considering interaction transfer energies and the related radiation species and their temporal development with application to biophysical models of radiation action.

Title of project no. :

Electron transport calculations, considering interaction transfer energies and the related radiation species and their temporal development with application to biophysical models of radiation action.

Head of project :

M. TERRISSOL

Scientific staff :

M. TERRISSOL, A. BEAUDRE, V. CAUDRELIER

I. Objectives of the project :

The aim of the research is to obtain spatial and time function distribution (10^{-16} to 1 second) of all chemical species involved within the slowing down of electrons and photons in biological material for energies up to 30 keV.

II. Objectives for the reporting period :

Improve the simulation code by comparisons with other experimental or theoretical works. Perfect the simulation of the thermalization of subexcitation and solvated electrons. Achieve the photon electron cascade down to about 10 eV in gazes and liquid water.

III. Progress achieved :

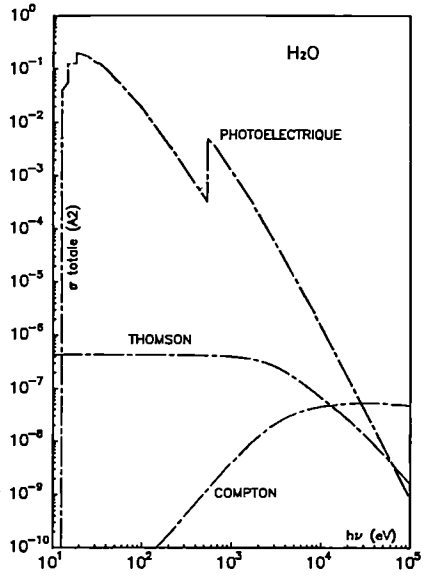
Our Monte-Carlo type code to simulate the physico-chemical stages following water radiolysis is now almost achieved. Using recent data on subexcitation electron transport, we have ameliorated the transport of these electrons and obtained interesting results on thermalization time and distance distributions. Comparing them with theoretic functions, we found that a modified exponential function applies for very low subexcitation energies (<2 eV), and a gaussian function for higher energies (up to 7.4 eV). But there is no valid function for the whole energy range.

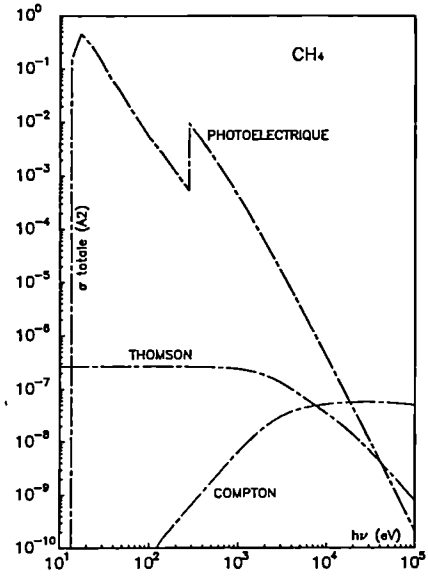
To simulate the photons transport in water and tissue-equivalent gaz, down to an energy of 10 eV, we must know all the molecular cross-sections and peculiarly the photoionization ones. Actual cross-sections found in literature have a lower limit of 100 eV and exist only partially for molecules, whatever the incident energy. We have studied this step during the past year.

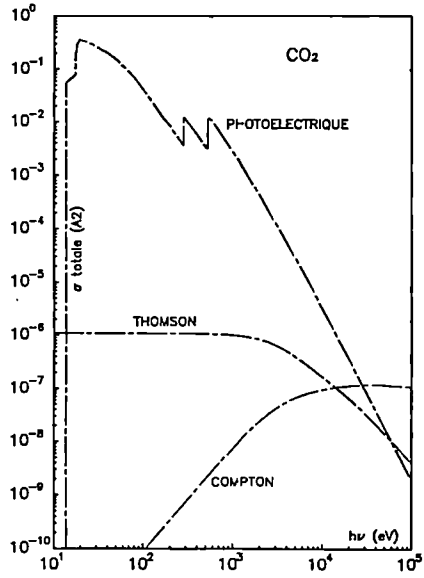
In the considered energy range (10 kev - 10 eV), classical interaction events are accompanied with complementary effects modifying the cross-sections. For the coherent and the incoherent scattering, we correct the THOMSON formula with the atomic structure factor and the KLEIN-NISHINA formula with the incoherent scattering function.

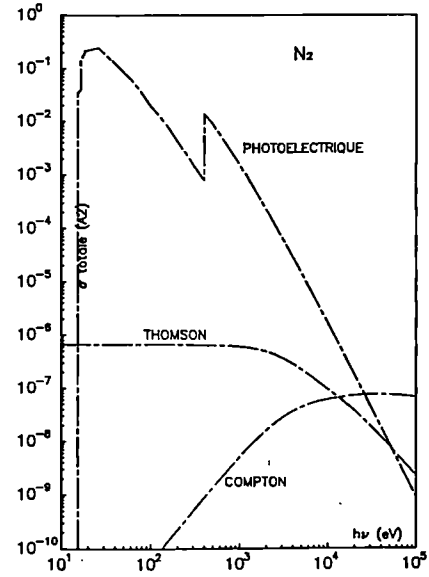
For the photoelectric effect and for energies near the ionizations thresholds, we use the differential cross-section given by RABALAIS. The complexity of molecular system make photoionization theory difficult to elaborate. It seems interesting to take into account several mechanisms that goes with each molecule. For total cross-sections, we have nevertheless adopted the semi-empirical summation theory of GELIUS, which allows to obtain each molecular orbital cross-section as a weighted sum of the corresponding atomic orbital cross-sections intervening in the molecule : the cross-section of one level is the sum of all atomic contributions to this level and that the intensity is proportional to the product of the cross-section by the electronic density.

There is a good agreement between found experimental results and our calculations, even at low energy and we used as far as possible all the available experimental data to calibrate our results. We can see on the next page, total cross-sections obtained for the H_2O, CO_2, CH_4 and N_2 molecules.


H_2O
PHOTOELECTRIQUE
σ totale (Å2)
THOMSON
COMPTON
hν (eV)


CH_4
PHOTOELECTRIQUE
σ totale (Å2)
THOMSON
COMPTON
hν (eV)


CO_2
PHOTOELECTRIQUE
σ totale (Å2)
THOMSON
COMPTON
hν (eV)


N_2
PHOTOELECTRIQUE
σ totale (Å2)
THOMSON
COMPTON
hν (eV)

IV. Objectives for the next reporting period :

Use of the physico-chemical code for radiochemistry, radiobiology or radiotherapy applications. Try to establish weighting rules in order to obtain photons results for gazes mixtures from separated ones.

V. Other research groups collaborating actively on this project [names and addresses] :

VI. Publications :

V. CAUDRELIER et M. TERRISSOL : " Les sections efficaces de quelques eV à 30 keV. " Compte rendus du 27ème Congrès de la Société Française des Physiciens d'Hôpitaux,Centre Jean Perrin, Clermont-Ferrand, Juin 1988. Résumé dans le Journal Européen de Radiothérapie, vol 3, 1988, Masson Ed.

A. BEAUDRE : " Simulation spatio-temporelle sur ordinateur des processus radiolytiques induits dans l'eau par des électrons. " Thèse n° 371, Doctorat de l' Université Paul Sabatier, Toulouse 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Radiobiological Institute TNO
Division for Health Research
Lange Kleiweg, 151
NL-2280 HV Rijswijk

Contract no.: BI6-A-002-NL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.J. Broerse
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Dr. J. Zoetelief
Radiobiological Institute TNO
Division for Health Research
Lange Kleiweg, 151
NL-2280 HV Rijswijk

Telephone number: 15-13.69.40

Title of the research contract:

Neutron dosimetry instrumentation for radiation protection and radiobiology.

List of projects:

1. Neutron dosimetry instrumentation for radiation protection and radiobiology.

Title of the project no.:

Neutron dosimetry instrumentation for radiation protection and radiobiology.

Head(s) of project: Prof.dr. J.J. Broerse and Dr. J. Zoetelief

Scientific staff: Prof.dr. J.J. Broerse, A.C. Engels, Dr. J. Zoetelief

I. Objectives of the project:

Studies on neutron dosimetry are required for radiation protection as well as for radiobiological investigations of mechanisms relevant for risk assessments. In radiation protection dosimetry there is still a need for the development of sensitive detectors with a response proportional to dose equivalent for a wide range of radiation qualities. For the interpretation of biological results obtained with various types of radiation, it is essential that dosimetry systems provide information on radiation quality in addition to accurate and precise dose values. The practical implementation of high-pressure ionization chambers for these purposes will be investigated.

II. Objectives for the reporting period:

The experimental studies with the TE and Al high pressure ionization chambers employing CH_4, Ar, TE-gas and C_2H_4 have been extended to neutron energies of 0.5 and 5 MeV. For these gases, the analysis of the pressure dependence of the reading, of ion recombination and of cavity size effects were continued. The information on cavity size effects is of importance to arrive at the saturation value of the readings at high-pressures at neutron energies below 15 MeV, which were studied initially. On the basis of the results obtained at 0.5 MeV a start has been made to investigate a practical high-pressure ionization chamber dosimetry system under actual conditions for radiation protection.

III. Progress achieved:

For the tissue equivalent high pressure ionization chamber irradiated with 5.3 MeV neutrons produced by the d(2.3)+D reaction, the relative readings (defined as the ionization chamber reading relative to that at 0.1 MPa and 600 V, denoted by $R(p,V_o)/R(p_o,V_o)$) as a function of the pressure of various gases are shown in the Figure. Up to about 5 MPa, the increase in the relative reading is proportional to the pressure for all gases investigated. Contrary to the situation for lower energy neutrons (i.e. 0.9 and 2.1 MeV, Zoetelief et al., 1985, 1988), there are no maxima observed in the relative readings as a function of gas pressure for CH_4 and TE-gas. The method to derive neutron energies from the value of the relative reading and the pressures at which the maxima are reached can not be applied with these gases for neutrons with energies of 5.3 MeV and can thus only be applied in a limited neutron energy range. For C_2H_4 a maximum is observed in the relative reading as a function of pressure at about 3 MPa, but the maximum seems very broad. Further studies employing C_2H_4 should be performed to evaluate the possibility of using the maxima for estimating neutron energies, in a large neutron energy range.
The pressure dependence of the relative reading with CH_4 for 5.3 MeV neutrons is very similar to that for 15 MeV neutrons and can not be used to determine neutron energy in this region. Employing TE-CH_4 gas in the TE chamber the plateau in the relative reading at 15 MeV neutron energy (i.e. 13) is only slightly higher than at 5.3 MeV (i.e. 11.8; see figure). The pressure dependence of the reading employing Ar shows considerable difference in the neutron energy range of 0.9 to 15 MeV and can be employed to derive radiation quality. However, at low neutron energies the relative reading is small (i.e. only about 3 at 6 MPa for 0.9 MeV neutrons) and consequently, the sensitivity of the dosimetry system is relatively small. In the Table the readings of the TE chamber with various gases relative to the reading with the TE-gas filled chamber at 0.1 MPa, are given for neutron energies of 0.9, 2.1, 5.3 and 15 MeV together with the recombination parameter R(500 V)/R(100 V) at 1 MPa. With regard to the sensitivity of the TE chamber filled with various gases, it can be derived from the figure and the table that the use of CH_4 provides the most sensitive system for 5.3 MeV neutrons. Concerning ion recombination, the use of TE-CH_4 gas seems most appropriate in the neutron energy range investigated, although also ion recombination in CH_4 can be used. The use of ion recombination in Ar is not appropriate for determination of radiation quality.

READINGS AND ION RECOMBINATION IN SEVERAL CASES FOR THE TE CHAMBER AT SEVERAL NEUTRON ENERGIES

	R(gas)/R(TE-CH_4) at 0.1MPa; 295 K; 600 V				R(500 V)/R(100 V) at about 1 MPa			
gas	0.9 MeV	2.1 MeV	5.3 MeV	15 MeV	0.9 MeV	2.1 MeV	5.3 MeV	15 MeV
Ar	0.54	0.88	1.04	1.02	1.15	1.13	1.06	1.05
TE-CH_4	1	1	1	1	2.26	1.56	1.42	1.29
CH_4	1.08	1.05	1.04	0.85	1.60	1.42	1.21	1.13
C_2H_4	-	1.46	1.33	-	-	1.60	1.39	-

References

- Zoetelief, J., Engels, A.C., Bouts, C.J., Broerse, J.J. and Hennen, L.A. 1985. Proc. Fifth. Symp., Neutron Dosim. EUR-9762. Vol. II, 705-715.
- Zoetelief, J. Golnik, N. and Broerse, J.J. 1988. Radiat. Protec. Dosim. 23, 451- 454.

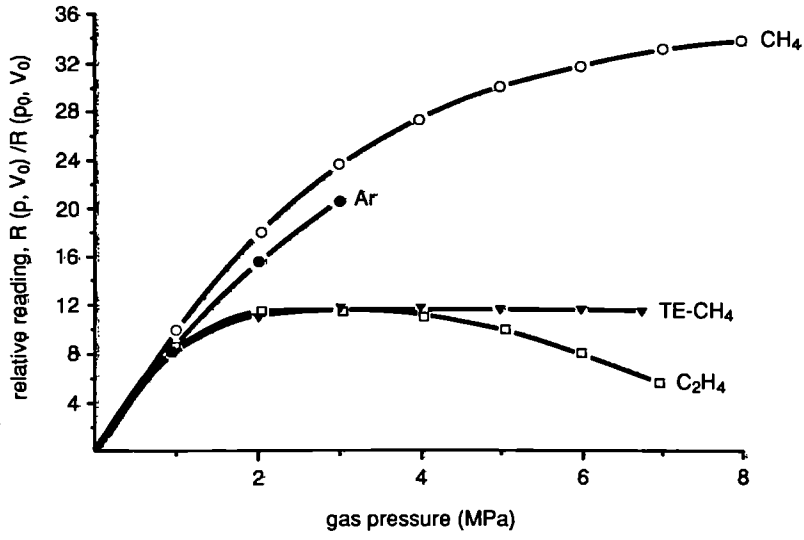

Figure. Relative reading of the TE high pressure chamber as a function of the pressure of various gases for 5.3 MeV neutrons.

IV. Objectives for the next reporting period:

The experimental studies with the TE and Al high pressure ionization chambers employing CH_4, C_2, H_4, TE gas and Ar will be continued for 0,5 MeV neutrons. The analysis of the pressure dependence of the reading, ion recombination and cavity size effects will be continued. This analysis will partly be performed in cooperation with the National Institute of Standard and Technology at Gaithersburg. The experimental studies of the high-pressure ionization chamber system will mainly be performed under conditions relevant for radiation protection practice.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Dr. J.J. Coyne, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA.
- Dr. N. Golnik, Institute of Atomic Energy, Swierk, Poland.

VI. Publications:

- Zoetelief, J Golnik, N. and Broerse, J.J. 1988,. Studies of high pressure ionization chambers in neutron and photon fields. Proc. Sixth Symp. on Neutron Dosimetry. Radiation Protection Dosimetry 23, 451-454.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

H.H. Wills Physics Laboratory
University of Bristol
Tyndall Avenue
GB Bristol BS8 1TL

Contract no.: BI6-A-006-UK

Head(s) of research team(s) [name(s) and address(es)]:

Prof. R.G. Chambers
H.H. Wills Physics Laboratory
University of Bristol
Tyndall Avenue
GB Bristol BS8 1TL

Dr. D.L. Henshaw
H.H. Wills Physics Laboratory
University of Bristol
Tyndall Avenue
GB Bristol BS8 1TL

Telephone number: 272-241.61

Title of the research contract:

A programme of study to examine the microdistribution of alpha-emitting radionuclides in man and the development of fast neutron spectrometry and dosimetry.

List of projects:

1. The microdistribution of alpha-active nuclides in the human lung.
2. A study of the uptake and burial of alpha-radionuclides in human bone.
3. The provision of facilities for the assay of occupationally exposed plutonium in lung, liver and skeleton.

Title of the project no.: B16-006-UK (2)

The microdistribution of α-active nuclides in the human lung.

Head(s) of project:

Dr D L Henshaw
Professor J E Enderby

Scientific staff:

Dr D L Henshaw
Dr A P Fews

I. Objectives of the project:

This project extends existing research in this laboratory using quantitative analysis of CR-39 α-particle autoradiographs. The lung burden of α-activity from particulate matter will be studied for the general population. The activity as a function of such factors as age and geographical location and smoking history will be studied. Application of the analysis techniques will enable the abundance of the principal α-active nuclides at each site and the identity of individual particles with multiple activity to be determined. The aim will be to use this information to elucidate the deposition, retention and clearance patterns of different α-emitting particles in association with their physical size and chemical form. In particular, the patterns of retention in both lymph nodes and tracheobronchial wall can be determined enabling proper microdosimetric calculations to be made.

II. Objectives for the reporting period:

For the studies in lung, the funding provided by CEC is limited to technical developments only and we expect the research in 1988 to be limited to this aspect. Our main research effort will be in human bone as outlined in a separate report.

III. Progress Achieved:

Within the funding provided, the largest effort during 1988 has been concentrated on studying alpha-emitting particles in human bone and bone marrow where substantial progress in our understanding has been made. A full report on our work in human bone appears under separate heading.

IV. Objectives for the next reporting period:

For the studies in lung, the funding provided by CEC is limited to technical developments only and we expect the research in 1989 to be limited to this aspect. Our main research effort will be in human bone as outlined in a separate report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

B16-006-UK (2)

Title of the project no.:
A study of the uptake and burial of alpha-radionuclides in human bone.

Head(s) of project:

Dr D L Henshaw
Professor J E Enderby

Scientific staff:

Dr D L Henshaw, Dr A Worley and Mr P H Randle

I. Objectives of the project:

In recent years new and sophisticated techniques for low-level α-particle autoradiography in CR-39 nuclear track detector have been developed in this laboratory. These techniques allow quantitative analysis of α-emitting particles in human tissue. The present study aims to use these techniques to fill important gaps in the scientific knowledge by providing a quantitative description of α-radionuclide uptake in human bone as a function of age, for the general population exposed to natural levels of activity. The work will include wherever possible parallel determinations of the α-radionuclide levels present in the liver which should provide new information on the rate of translocation to the skeleton in man.

II. Objectives for the reporting period:

The year will be dominated by further studies of α-activity in human bone and red marrow. In particular the aim will be to measure the α-doses in the red marrow of children as a function of age. At the same time studies will continue in the current tissue under storage. Here detailed investigations will be made in whole femur and the vetebral column.

III. Progress achieved:

Methodology In our previous report we gave details of our analysis of alpha-particle autoradiographs of autopsy bone samples from adults and children in West Cumbria, UK. The results implied that contrary to the standard ICRP model for bone, the ^{210}Po produced by decay of ^{210}Pb in bone appears to be released from bone and is free to migrate into both marrow and surrounding tissue. The resulting alpha-radiation dose to red marrow was shown to be $\sim$800 $\mu Sv.y^{-1}$, similar to that from low LET sources.

As an extension to this work we have recently performed a separate analysis of bone marrow and muscle adjacent to the femur. The fatty, yellow marrow was employed extracted from the shaft of the femur.

As a separate piece of work we have considered, theoretically, the radiation dose from radon exposure to organs other than lung, in particular to red marrow and to the fetus.

Results

1. Pure Marrow Measurements

The results of the pure marrow analyses are presented in Table 1. All of the activity present was in the form of single alpha-decays on the plastic autoradiograph and we assume to be due to the decay of ^{210}Po. If we assume that the activity in marrow is in fact supported by ^{210}Pb in marrow, then we obtain the uncorrected activities indicated. These themselves are lower than the average for the previous cases but still well above what would be predicted by the standard ICRP model for bone. If, on the other hand, we assume the activity is due soley to ^{210}Po in marrow of half life 135 days, then we must apply the following correction to the time integrated count on the plastic:

$$\text{corrected activity} = \frac{T2}{(C2 - C1)} \times (\text{raw activity})$$

Where $C1 = 195(1- e^{-0.693T1/135})$ and $C2 = 195(1- e^{-0.693T2/135})$

and T1 is the time interval between death and mounting of tissue against the CR-39 autoradiograph and T2 is the time interval between death and unloading the autoradiograph.

This correction has been used to derive the corrected activities indicated. The dose values corresponding to these measurements are given in Table 2 and are well in excess of what would be assumed by the standard ICRP model for bone and indeed greater than that calculated from low LET sources.

Table 1 Summary of Marrow and Muscle Activities

Case	Region	Storage Time (days)	Sample Area (cm^2)	Number of Tracks	Activity ($Bq.kg^{-1}$) Uncorrected	Corrected
148	Marrow	397	83.7	794	0.20 +/- 0.01	1.17 +/- 0.06
152	Marrow (1)	237	24.8	220	0.20 +/- 0.03	2.78 +/- 0.42
"	" (2)	237	24.8	170	0.30 +/- 0.03	4.17 +/- 0.42
"	Muscle (1)	237	10.0	88	0.29 +/- 0.05	4.03 +/- 0.70
"	" (2)	237	10.0	86	0.28 +/- 0.05	3.89 +/- 0.70

Table 2. Dose Values from Separate Marrow Measurements

Case	Region	Correction Factor	Red Marrow Dose $\mu Sv.y^{-1}$
148	Marrow	5.85	107 - 626
152	Marrow (1)	13.90	107 - 1487
"	" (2)	13.90	160 - 2224
"	Muscle (1)	13.90	155 - 2155
"	" (2)	13.90	150 - 2085

Note The range of dose values encompasses the difference between using activity values either uncorrected or corrected for pure ^{210}Po decay.

2. Radon Dose to Organs other than Lung

The risk from radon has to date been discussed solely in terms of the risk of induction of lung cancer. Whereas the lung undoubtedly receives the highest dose from inhaled radon and its short-lived daughter nuclei, other body tissues, including the bone marrow of all age groups and the developing fetus, must also receive a radiation dose either from radon itself, its short-lived or its long-lived daughter nuclei. Therefore it is pertinent not only to determine the natural alpha-radiation dose received by the fetus, but, to what extent this is influenced by environmental

alpha-radiation especially from radon.

The factors governing the radiation dose to lung from inhaled radon and its short-lived alpha-emitting daughter nuclei have been well investigated (NEA Experts Report 1983). The kinetics of radon and radon daughter products in the body may be described as four processes (Jacobi and Eisfeld 1980).

1) The uptake from air into blood
2) The convective transport into the bloodstream
3) The diffusion from blood into intracellular fluids
4) The radioactive decay during the transport in the body

We propose that the important pathways governing the alpha-radiation dose to red marrow and to the fetus may be represented diagramatically as in figure 1 (a) and (b). Pre-natal irradiation can take place from the transfer of inhaled radon and its short-lived daughter nuclei and from the transfer of the so-called radon long-lived daughter nuclei ^{210}Pb and ^{210}Po, accumulated in the skeleton of the mother from her lifetime radon exposure. Post-natal irradiation of the red bone marrow in the growing child is derived partly from inhaled radon and its short-lived daughters and partly from ingrowth of the long-lived daughters of radon. All other nuclides such as naturally occurring ^{226}Ra and fallout plutonium provide a negligible contribution to the total dose received.

We will now consider the relevant pathways indicated in figure 1.

Radon

Radon activity in the body has been estimated from published solubility values (See for example Peterman and Perkins 1988). Although such estimates indicate that only a small proportion of inhaled radon enters the bloodstream in cases of high radon exposure this will nevertheless lead to significant doses to tissues such as red marrow and to the fetus. However, we question whether simple solubility data may be applied in this way to the lung in-vivo. In a recent pilot experiment we exposed water, with and without stirring, to a known radon atmosphere. Subsequent determination of radon activity in the water showed values 9 times higher in the stirred (analogous to blood flow) compared with the static solution. Given that the surface area of the lung alveoli is some 45 m^2 with blood flow along the alveolar walls, we suggest that the fraction of inhaled radon that enters the bloodstream is unknown, that the fraction shown in the first path in fig 1 (a) is unknown and that its determination represents a significant experimental problem.

Although direct measurements of radon in the mother and fetus have not been made it is known that another inert gas ^{85}Kr freely crosses the placenta of animals in both directions and its concentration is the same in maternal and fetal blood (Sikov 1988). Uptake characteristics and concentrations in

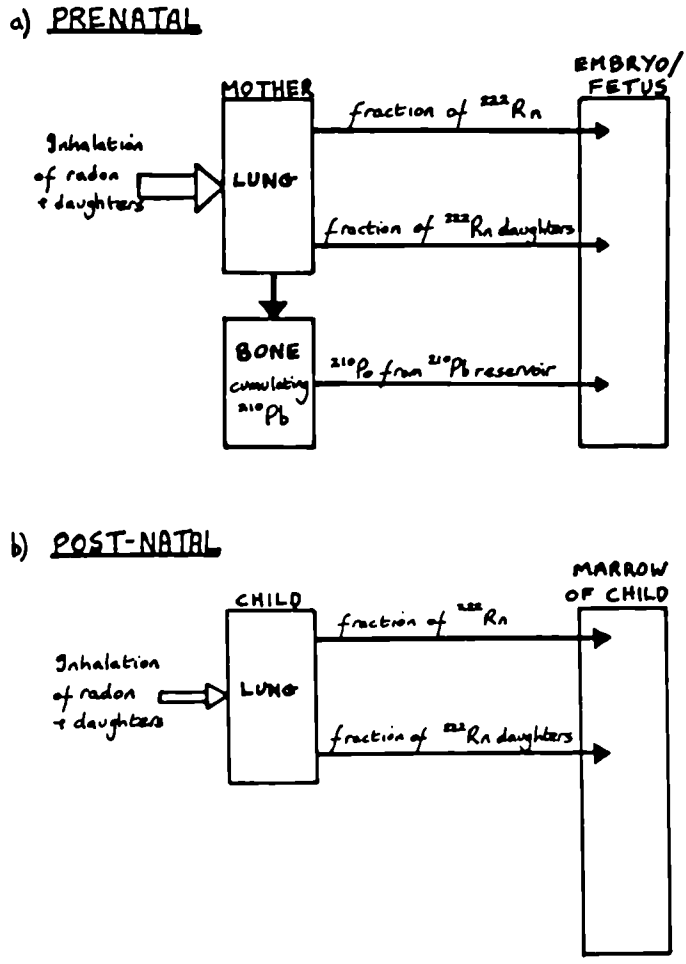

Figure 1 The principal routes by which alpha-emitters reach and irradiate embryo and fetus.

Pre-natal (a): Short term variations of alpha-activity from the mothers inhaled radon and its short-lived daughter products. Only a small fraction of radon and daughter products pass to the embryo yolk sac, fetal liver and bone marrow, which are target organs for radiation induced leukaemia. The embryo and fetus may also be irradiated by alpha-emitting ^{210}Po from the mother's long term cumulative reservoir of ^{210}Pb.

Post-natal (b): At birth, the neonate is subject to alpha-radiation from its own radon gas inhalation, at a slower rate than the mothers, with a small fraction of radon and decay products irradiating the stem cells that reside in bone marrow. The child's own reservoir of ^{210}Pb then accumulates.

fetal tissues would be expected to be similar to those of adults.

Short-lived alpha-emitting daughters, ^{218}Po and ^{214}Po.

With a physical half life of 3.8 days, ^{222}Rn decays to its short-lived alpha-emitting daughter products ^{218}Po and ^{214}Po with half lives less than 30 minutes. These short-lived daughter nuclides originating from the decay of ^{222}Rn either in the lung or in the bloodstream may also be partly transferred to body tissues including the red marrow and the fetus.

Long-lived daughters ^{210}Pb and ^{210}Po.

Uranium miners provide the opportunity to study at autopsy the levels of ^{210}Pb and ^{210}Po in the skeleton following protracted inhalation of radon in the mine atmosphere. One such study in rib and veterbra was by Blanchard, Archer and Saccomanno in 1969. For these bones the relationship between ^{210}Pb concentration and the cumulative exposure was found to be non-linear as indicated in figure 2. The ^{210}Pb concentration in rib is plotted as a function of cumulated exposure in Working Level Months, WLM where an exposure of 0.1 WLM per year is equivalent to an exposure of 20 $Bq.m^{-3}$ per year (Wrixon et al, 1988).

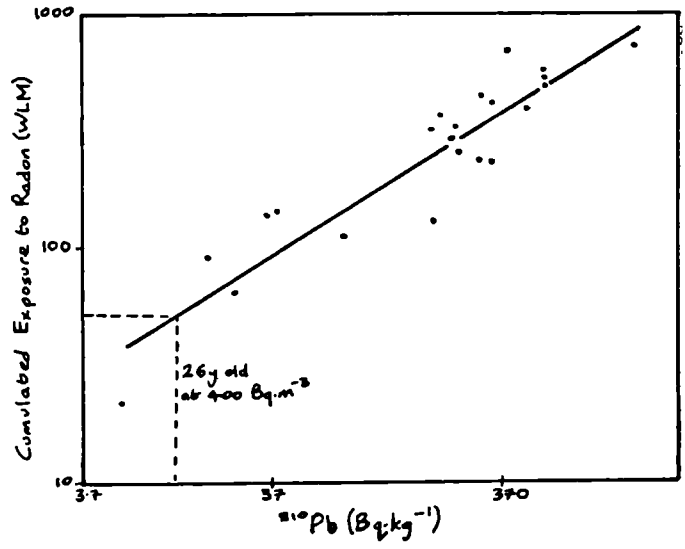

Figure 2 Data of Blanchard et al, 1969 showing the ^{210}Pb content of bone in workers after cumulative exposure to radon and its short-lived daughters in uranium mines.

These data have been used to calculate the ^{210}Po content of bone for persons of different ages living in houses at given radon concentrations. The ^{210}Po content in bone after cumulative exposure to radon and radon daughter products at 20, 400 and 1000 $Bq.m^{-3}$ are given in Table 3. Also given in the table, in brackets, is the dose equivalent to bone in $mSv.y^{-1}$. In view of the evidence that ^{210}Po produced by the decay of ^{210}Pb in bone is released from bone (Henshaw et al 1988) we suggest that these dose values also apply to red bone marrow. Calculations have been made for two representative ages; age 10y for the growing child representing a cumulative dose as in figure 1 (b) and age 26y the average age of conception of women in Europe, representing a dose to the mother in its own right a and source of ^{210}Po which may be transferred to the fetus.

Table 3. Predicted ^{210}Po content in bone from cumulative exposure to radon and daughter products.

Radon $Bq.m^{-3}$	Cumulative Exposure WLM		^{210}Po content $Bq.kg^{-1}$ ($mSv.y^{-1}$ to red marrow)	
	10y	26y	10y	26y
20 (UK ave)	1.0	9.6	0.009 (0.004)	0.044 (0.023)
400 (UK Action Limit)	20	52	1.3 (0.69)	6.4 (3.4)
1000 (Some Houses in SW England)	50	130	6.0 (3.2)	30 (16)

Note that for average radon concentrations, 20 $Bq.m^{-3}$, the predicted ^{210}Po bone content is considerably below that measured experimentally (Henshaw et al 1988, Czegledi 1977) so it must be assumed that there is a component of $^{210}Pb/^{210}Po$ from dietary intake. Given that there was no reason to suppose that the samples analysed were from former residents of houses with high radon exposure, we propose that the high doses calculated for continuous exposure to 1000 $Bq.m^{-3}$, that is 3.2 and 16 $mSv.y^{-3}$ at ages 10y and 26y respectively, represent realistic estimates of bone marrow dose attributable to radon. We emphasize, however, that these doses represent only one of the pathways in figure 1 and that there must be additional dose contributions from radon itself and its short-lived daughter nuclei for which no data exists at present.

There is evidence in humans confirming the cumulative effects of exposure to inhaled radon and its short-lived daughters in domestic dwellings. Higher ^{210}Pb levels were found in the teeth of Austrian spa workers exposed to elevated radon levels compared to an Italian population inhaling "normal" levels of radon and radon daughters (Clement et al 1984).

An increase in the alpha-activity body burden with age would be expected from a cumulative effect to exposure to radon gas. Higher levels of ^{210}Pb and ^{210}Po have been found in older age groups relative to younger ones. (See Table 4.)

Table 4. Comparison of ^{210}Pb and ^{210}Po levels in the human bone of different age groups.

Authors	Study Groups	Samples	Nuclide	Age Ratio of groups	Activity Ratio
Czegledi 1977	infants/ adolescents	total skeleton	^{210}Pb	13	10
			^{210}Po	13	11
Henshaw 1988	children/ adults	bone/ marrow	^{210}Po	7	4.5

It is not known whether higher incidences of childhood leukaemia result from parents, especially the mother, who reside in homes with elevated radon levels. The alpha-activity body burden can be expected to be greater for residents of dwellings with high radon levels. Epidemeological evidence for the association of leukaemia with high levels of radon inhalation will be difficult to obtain due to variability of radon levels in houses even in the same neighbourhood (NRPB-R190, 1988) and may only be productive with individual assessment of domestic radon levels. Other factors such as the older mother and smoking habits may also increase the alpha-activity body burden of ^{210}Po and result in an increased incidence of childhood leukaemia.

In addition to radon there is a contribution to bone marrow dose from radon-220 (^{220}Rn) or thoron produced in the thorium decay chain. Environmental thoron reaches the lung in much smaller quantities compared with radon owing to its short half-life of 55 seconds. A major part of the derived dose from thoron is from the daughter lead-212 (^{212}Pb) which has a half-life of 10.4 hours (James 1988).

Discussion

1. Pure Marrow Measurements

The doses to red marrow calculated in Table 2, attributable to radon exposure, are, in some cases significantly higher than that from other (low LET) sources of radiation, from terrestrial gamma rays and sea-level cosmic rays. They represent an underestimate of the dose received in-vivo as they take no account of the contribution from radon itself and its short-lived daughter nuclei. This suggests that if we believe that environmental radioactivity is implicated in the incidence of childhood leukaemia then epidemeological studies should be concentrated on areas of high radon exposure.

2. Radon Dose to Marrow Fetus

Direct measurement of alpha-activity in fetal tissues and placenta is required (a) in its own right to determine typical dose values in the general population; (b) in order to determine the transfer factor from the placenta to the fetus; (c) in order to correlate the levels present with radon exposure, with maternal age and smoking history and (d) so that any association with regional clusters of childhood cancer may be investigated.

We have started a programme of measuring alpha-activity in fetal tissues and corresponding placenta and we expect first results to be available towards the end of 1989.

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Wrixon A. B., Green B. M. R., Lomas P. R. et al "Natural radiation exposure in UK dwellings", NRPB R190 HMSO, London. (1988).

IV. Objectives for the next reporting period:

Measurements will continue of alpha-radiatin doses to red marrow and of fetal transfer of alpha-activity at natural levels of exposure.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Hatzialekou U., Henshaw D. L. and Fews A. P. "Automated Image Analysis of Alpha-particle Autoradiographs of Human Bone" Nucl Instr Meths A263, 504-514 (1988)

Henshaw D. L., Hatzialekou U. and Randle P. H. "Analysis of Alpha Particle Autoradiographs of bone samples from adults and children in the UK at Natural Levels of Exposure" Radiation Protection Dosimetry Vol. 22, No. 4 pp 231-242 (1988).

B16-006-UK (3)

Title of the project no.:

The provision of facilities for the assay of occupationally exposed plutonium in lung, liver and the skeleton.

Head(s) of project:
Dr D L Henshaw
Professor J E Enderby

Scientific staff:

Dr D L Henshaw

I. Objectives of the project:

Much of this work will involve the analyis of α-activity by CR-39 autoradiography in autopsy samples for litigation purposes. Therefore, the measurements obtained will not in general be available for publication.

The objective of the project, vis-a-vis the CEC funding provided, is to construct new laboratory facilities and in due course gain approval by the Health and Safety Executive (UK) for this work. The analyses should use techniques proven in the related research at Bristol on uptake of α-radionuclides by man.

II. Objectives for the reporting period:

The objective was to analyse such cases as became available through the National Radiological Protection Board (UK) where existing collaboration exists. Work should commence on a purpose-built laboratory in the Physics Department at Bristol University.

III Progress achieved

It is not been possible to do meaningful work on this project during 1988. One sample has become available and this is currently under storage to be analysed within the next reporting period. We have continued to analyse natural alpha-activity in bone and marrow and the results of this work are the subject of a separate report.

The building of a purpose laboratory for tissue handling has now been approved and construction work is due to commence during 1989.

IV. Objectives for the next reporting period:

Notwithstanding the lack of tissue samples available in 1987, the analysis of any samples that become available in 1988 can proceed. The building of a purpose laboratory for tissue handling has now been approved and construction work is due to commence during 1989.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

(1) National Radiological Protection Board, Harwell UK.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Com.Naz.per la Ricerca e per lo
Sviluppo dell'Energia Nucleare e
delle Energie Alternative, ENEA
Viale Regina Margherita, 125
I-00198 Roma

Contract no : BI6-A-004-I

Head(s) of research team(s) [name(s) and address(es)]:

Prof. M. Coppola
Div.Fisica e Scienze Biomed.
ENEA, CRE Casaccia
Casella Postale 2400
I-00100 Roma

Telephone number: 6-30.48.39.64

Title of the research contract:

Study of radiobiological effects at low doses.

List of projects:

1. Study of radiobiological effects at low doses.

Title of the project no.: 1

Study of radiobiological effects at low doses.

Head(s) of project:

Dr. Vincenzo Covelli

Scientific staff:

Prof. M. Coppola, Dr. V. Di Majo, Dr. S. Rebessi

I. Objectives of the project:

Study of the biological effectiveness of low doses of different radiation qualities for various modes of irradiation and suitable endpoints (life-shortening and tumour induction) in experimental animals.

A. Analysis of results obtained from large experimental series on mouse populations whole-body irradiated with low doses of neutrons and X rays (single and fractionated), for the study of dose-effect relationships for tumour induction in different organs at risk.

B. Study of in vivo risk of transformation per cell by an experimental model system based on transplant of irradiated cell suspensions into syngeneic hosts. Results to be compared with data for irradiated intact animals.

II. Objectives for the reporting period:

Analysis of data of tumour induction in mice irradiated with low doses of neutrons and X rays.

Continuation of the follow-up of mice irradiated with fractionated doses of fission neutrons and X rays.

Further investigations on the risk of neoplastic transformation per cell in epithelial tissues.

III. Progress achieved:

The main contractual activity in 1988 was devoted to the continuation of in vivo studies of the dose-response relationships for late somatic effects induced by either sparsely or densely ionizing radiation, distinctively at low doses. In particular, the attention was concentrated on 1) the analysis of data of life shortening and tumour induction in irradiated mice for the evaluation of stochastic effects (tumours and leukaemias), non-stochastic effects (degenerative disease) and of their variation in relation to various physical factors, such as radiation quality, beam energy, dose and irradiation mode, 2) the follow up and the collection of data of survival and pathology at death in mice exposed to several doses of fast neutrons and X rays.

Complete observation of survival and late pathology was begun over a population of about 2000 $BC3F_1$ male mice, which had been irradiated with fractionated doses of fission neutrons, produced by the RVS TAPIRO reactor of ENEA Casaccia, and followed until spontaneous death. 9 different mouse groups were given 5 equal daily dose fractions of 0.5 to 14.2 cGy, for total doses from 2.5 to 70 cGy, at a dose rate of 0.41 cGy/min. A similar fractionated dose irradiation was carried out using X rays, for total doses from 25 to 300 cGy. This experiment appears very promising as it will allow the in vivo investigation of the influence of dose fractionation on late somatic effects.

Tumour induction has been studied in mice irradiated on the lymphatic system with a fractionated protocol (TL1). A similar protocol is utilized in medicine for the treatment of patients with lymphoma or which have to undergo an organ transplantation. In particular, using a mouse population with a high natural lymphoma incidence, it was intended to investigate whether the expected depression of this tumour following irradiation is associated with the appearance of other tumour types or the life span shortening. The results indicate the presence of a relevant incidence of

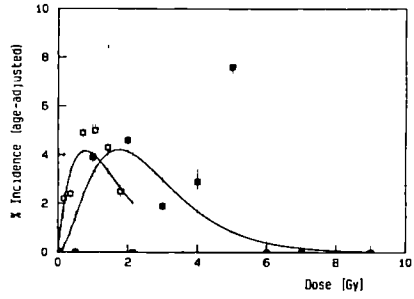
	No. of mice	Mean survival time ± SD (days)	Nephro-sclerosis	Malignant lymphoma	Solid tumors					
					Lung	Liver	Skin	Soft tissues	Others	Total
Control	88	887 ± 189	7 (8)	43 (49)	5 (6)	12 (14)	1 (1)	4 (5)	4 (5)	26 (30)
Irradiated	68	673 ± 144	26 (38)	4 (6)	3 (4)	13 (19)	18 (26)	5 (7)	6 (9)	45 (66)

malignant skin cancers, which appear to be the most likely cause of life shortening of the treated mice, together with kidney degenerative disease (see Table).

The analysis of lymphoma and myeloid leukaemia incidence data in about 3000 $BC3F_1$ male mice irradiated in Casaccia with single acute doses of X rays and fission neutrons has pointed out some interesting aspects regarding the shape of the dose-effect relationships. For malignant lymphoma, which shows a very high spontaneous incidence, the data are well described assuming for X rays a quadratic-dose induction model and for fission neutrons a linear model, corrected by exponential cell inactivation operating on both the spontaneous and the radiation-induced components. The best fitting value for the inactivation probability per unit dose was 0.70 ± 0.04 Gy^{-1} and 1.0 ± 0.5 Gy^{-1} for X rays and fission neutrons, respectively. Myeloid leukaemia in $BC3F_1$ mice is a rare event, nevertheless, the dose-response curves are very similar in shape to those already reported for CBA/H and RF/Un mice. Also for this tumour, the induction appears to be linear for fission neutrons and quadratic for X rays, with an exponential correction for cell inactivation (see Figure).

The feasibility of new experimental series to study the risk of neoplastic transformation both in vivo and in vitro was studied. In particular, preliminary measurements have been performed on the production of transforming clones in cell cultures irradiated with different radiation qualities .

Further experimental work was also carried out, in a wider collaboration effort, during 1988 to collect information on the influence of the damaging agent (radiation and/or chemicals) on the relative yield of different types of chromosomal aberrations in human lymphocytes.



Myeloid leukaemia induced by 250 kVp X rays (full squares) and fission neutrons (open squares) in $BC3F_1$ mice.

IV. Objectives for the next reporting period:

Analysis of data of tumour induction in mice after total lymphoid irradiation with single acute doses of X rays.

Continuation of the follow-up of mice irradiated with low fractionated doses of fission neutrons and X rays.

Further investigations of the risk of neoplastic transformation per cell in vivo and in vitro.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

1) Coppola, M., Covelli, V., Di Majo, V., Rebessi, S. Study of dose-response relationships for late somatic effects of low neutron doses. In: Neutron Dosimetry, Proceedings of the Sixth Symposium on Neutron Dosimetry (H. Schraube, G. Burger, J. Booz, eds.). Radiation Protection Dosimetry 23, Nos 1-4, 69-72, 1988.

2) Covelli, V., Coppola, M., Di Majo, V., Rebessi, S., Bassani, B. Tumour induction and life shortening in $BC3F_1$ female mice at low doses of fast neutrons and X rays. Radiat. Res. 113, 362-374, 1988.

3) Di Majo, V., Covelli, V., Coppola, M., Rebessi, S., Bangrazi, C., Bassani, B. Long-term effects in mice after Total Lymphoid Irradiation. 79th Annual Meeting of the American Association for Cancer Research. Cancer Res, New Orleans, Louisiana, 1988.

4) Covelli, V., Di Majo, V., Coppola, M., Rebessi, S., Bangrazi, C., Doria, G. Late somatic effects in mice after total lymphoid irradiation (TLI). Radiat. Res., 1988. (in press)

5) Di Majo, V., Covelli, V., Coppola, M., Rebessi, S., Bangrazi, C. Depression of malignant lymphoma in $BC3F_1$/Cne mice following Total Lymphoid Irradiation (TLI). International Meeting on physical, biological and clinical aspects of total body irradiation, Den Haag, The Netherlands, 1988.

6) Covelli, V., Coppola, M., Di Majo, V. Current studies on experimental radiation carcinogenesis. In: Radiation Protection: Advances in Yugoslavia and Italy. Proceedings of the II Yugoslav-Italian Symposium, Udine, Italy, 1988.

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8) Covelli, V., Coppola, M., Di Majo, V., Rebessi, S. Experimental models for radiation carcinogenesis. 14th L.H. Gray Conference, Oxford, U.K., 1988.

9) Coppola, M., Bertoncello, G. Neutron RBE for the production of micronuclei in the eye-lens of irradiated mice. (Submitted)

10) Covelli, V., Di Majo, V., Coppola, M., Rebessi, S. The shape of the dose-response relationship for myeloid leukemia and malignant lymphoma in $BC3F_1$ mice. (Submitted)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Istituto Nazionale di
Fisica Nucleare
Sede Centrale INFN
Casella Postale 56
I-00044 Frascati / Roma

Contract no.: BI6-A-193-I

Head(s) of research team(s) [name(s) and address(es)]:

Prof. P. Dalpiaz
Laboratori Nazionali dell'
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Via Romea 4
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Dr. P. Colautti
Laboratori Nazionali dell'
INFN di Legnaro
Via Romea 4
I-35020 Legnaro (Padova)

Telephone number: 049/641.200

Title of the research contract:

Stochastic variables in the energy deposit and their meaning in the hazard of neutrons.

List of projects:

1. Stochastic variables in the energy deposit and their meaning in the hazard of neutrons.

Title of the project no.: BI6-A-193-I

Stochastic variables in the energy deposit and their meaning in the hazard of neutrons

Head(s) of project:

Dr. P. Colautti

Scientific staff:

P. Colautti
G. Talpo
G. Tornielli

I. Objectives of the project:

The project concerns with the study of the stochastic variable "y" (lineal energy) at simulated diameter less than 1 μm.

Objective of the project is to investigate the possibility to manufacture a spherical tissue-equivalent proportional counter able to work properly at pressures as low as possible in order to simulate tissue diameters less than 1 μm.

II. Objectives for the reporting period:

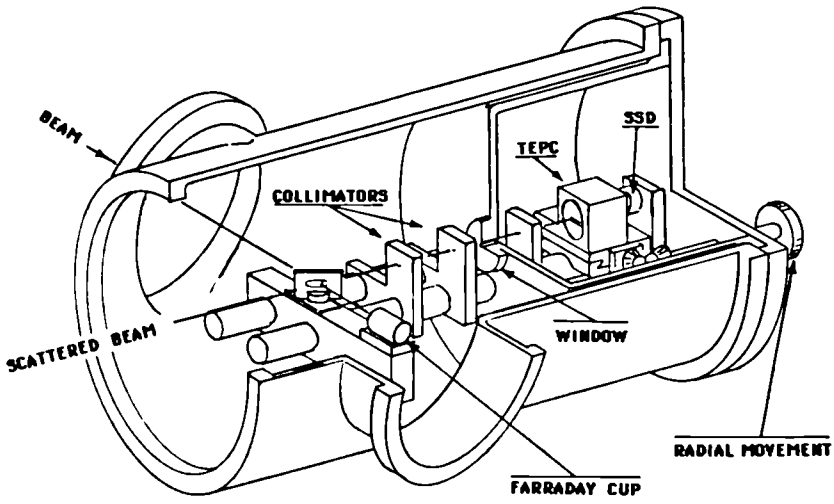
The experimental work had to be devoted to the study of the working characteristics at low pressure of a tissue-equivalent proportional counter in order to investigate the limits of this technique to measure the energy absorbed in simulated diameter less than 1 μm.

III. Progress achieved:

In the ordinary use of a tissue-equivalent proportional counter (TEPC) one can observe that the most severe limit to the simulation of diameters less than 1 μm is due to the decreasing of the gain with the gas pressure so the electrical signals due to the energy deposition events decrease while the electrical noise remains unchanged.

Studies performed in the last seventies with multiwires proportional counters showed an unexpected high multiplication factor at low pressure (about 1 torr or less) explained with an increase of the inelastic collision probability at high values of the reduced electrical field (E/p) and with the consideration that the amplification starts at a large distance from the wire. Further studies showed that when the mechanism of the avalanche formation is divided into two steps high gains (10^5-10^6) are reached.

A measurement chamber has been projected and manufactured to study the work limits at low pressure of a TEPC (see figure).



A scattered beam of light ions enters into the detector parallely to the anode through a thin window; the beam is employed as a signal probe, it scans the region between the anode and the cathode in order to study the gain characteristics and the multiplication region limits of the detector.

The anode and grid structure, which is more suitable to obtain high gains at low pressure, will be studied and then it will equip a 2.5 cm spherical TEPC which will be exposed at dose-calibrated neutron beams to study the experimental change of neutron microdosimetric spectra in decreasing the simulated diameter. The facility to produce collimated neutron beams has been completed and the dosimetric and microdosimetric characteristics, at 1 μm simulated diameter, of a d(4.5)+Be neutron beam have been determined.

IV. Objectives for the next reporting period:

The experimental set-up will be assembled. Measurements with α-sources and accelerated ion beams will be performed.

The experimental results and the comparison with calculated data will point out the building characteristics of a TEPC which is able to measure at simulated diameters less than 1 micrometer.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. H. Schraube and Dr. G. Leuthold
Institut für Strahlenschutz FSF - München - FRG

Dr. D.E. Watt
Physics Department of the St. Andrews University - U.K.

VI. Publications:

P. Colautti, G. Talpo and G. Tornielli
"A facility to produce collimated neutron beams at the Legnaro Laboratories"
Radiation Protection Dosimetry 23, 321-324 (1988).

P. Colautti, G. Talpo, G. Tornielli, H. Schraube, G. Leuthold, D.E. Watt
"Stochastic variables in the energy deposit and their meaning in the hazard of neutrons"
LNL Annual Report 1987, LNL-INFN (REP)-014/88.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université de Limoges
Allée André Maurois
F-87060 Limoges Cédex

Contract no.: BI6-A-192-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.L. Decossas
L.E.P.O.F.I.
123, rue Albert Thomas
F-87060 Limoges Cédex

Dr. J.C. Vareille
L.E.P.O.F.I.
123, rue Albert Thomas
F-87060 Limoges Cédex

Telephone number: 55.45.74.51/55

Title of the research contract:

Study and realization of a high performance personal neutron dosemeter.

List of projects:

1. Study and realization of a high performance personal neutron dosemeter.

Title of the project no. : B 16 - A - 192 - F

Study and realization of a high performance personal neutron dosemeter

Head(s) of project :

Dr. J.L. DECOSSAS
Dr. J.C. VAREILLE

Scientific staff :

Dr. DECOSSAS, MAKOVICKA, VAREILLE

I - Objectives of the project :

Calculation of dosemeter responses for neutrons, realization of a composite radiator detector system based on CR 39 and experimental test of the developped dosemeter.

II - Objectives for the reporting period :

- tests and calibrations of our automatic tracks counting and analysing system (BIOCOM IMAGENIA),

- application of this device to investigations on a dosemeter background,

- test of the "multidetector dosemeter" [Bore doped $(CH_2)_n$ through the differential method in field of rapid and thermal neutrons.

III - Progress achieved :

1 - Methodoly

An image analysis system (BIOCOM IMAGENIA) was used to analyse and count the tracks in our etching conditions ; the following methods and criteria were used :

- the conversion tables were modified to enhance the contrast,
- pits with an area $\leq 5\ \mu m^2$ were not counted,
- the form factor pits, defined as $F = 4\Pi S/p^2$ (S being the surface and p the perimeter) was $F \geq 0,65$.

The system was tested.

* polyéthylène was implanted with Bore on HVEE accelerator with fluences about 1,7 10^{15} atomes cm^{-2},
* irradiations were made at SIGMA in CEN CADARACHE (thermal neutrons) or CEA FONTENAY-AUX-ROSES (3 MeV neutrons),
* DST were etched by NaOH - 6 N 60°C during 14 hours.

2 - Results

a - Background on dosemeter built with Pershore CR 39
(age of CR 39 : 1.5 months)

Results are summarized in table 1. Sample n°1 is analyzed by the automatic system with the above criteria. For sample n°2 and 3, the doubtful tracks are discreminated. We can note that the response is better in the second case. The best one is obtained when B = 0,37 mSv and S = 0,444 mSv. The background (in mSv) depends on the radiator thickness and obviously on CR 39 quality and the pits analyzed.

Furthermore, preetching is able to reduce background, for example with Am Be irradiation :

- sample without preetching : signal / background # 1,6
- sample with preetching : signal / background # 2,9

(60% ethanol + 40% NaOH 6,25 N ; 70°C, 1 hour)

Table 1

Sample n°	Polyethylene radiation thickness	Sensitivity (*) $cm^{-2}\mu Sv^{-1}$	Highest b μSv^*	Highest B μSv^*	Highest b-B μSv^*	Highest S μSv^*
1	150 µm	1.23	2693	1506	1457	924
2	150 µm	1.23	1303	369	934	444
3	135 µm	0,36	4450	1261	3189	1520

* Convert tracks cm^{-2} to µSv using the sensitivity given in column 3
(*) Reference is that of 3 MeV neutrons

Meaning of symbol
µSv : the corresponding H'(10) of Calibration Neutrons
b : Background Reading of an Individual Sample (µSv)
B : Average of b for a Sheet (µSv)
S : Standard Deviation of b for a Sheet (µSv)

b - Test of our dosemeter by means of the image analysis system

It was done for 3.3 MeV neutrons with different radiator thicknesses (20 µm, 35 µm, 150 µm). Results are summarized in figure n°1.

c - Test of the multidetector dosemeter in a field of thermal neutrons

It is based on the differential method and albedo is taken into account. Phantom is realizedwith polyethylene and water. At SIGMA, we worked with a dose equivalent equal to 33.4 mSv (thermal neutrons). The device is described in figure n°2.

In the above conditions, we obtained :

- R_A = 31500 tracks cm^{-2}
- R_B = 10500 tracks cm^{-2}
- R_C= 17750 tracks cm^{-2}
- R_D = 6250 tracks cm^{-2}

So, we can obtain :

$R_A - R_C$ = Thermal neutron response, in this case $R_A - R_C$ = 13750 tracks cm^{-2} which correponds to 27,5 mSv (dosemeter sensitivity : 500 tracks mSv^{-1}).

$R_B - R_D$ = fast neutron response

By means of albedo and through a calculation which is more difficult than the above one, we can obtain the response for intermediary neutrons.

3 - Dis cussion

By means of the image analysis system, we obtained a large field of results in order to improve our statistical study of DST (CR 39) background. From table 1, we can see that the optimal thickness (35 µm) for a flat response versus neutron energy is not the best for background.

We noted that it is necessary to have a better discrimination of doubtful tracks, so we must improve the criteria chosen for the automatic system.

The first tests of the "multidetector dosemeter" are in rather good agreement with our previous calculations.

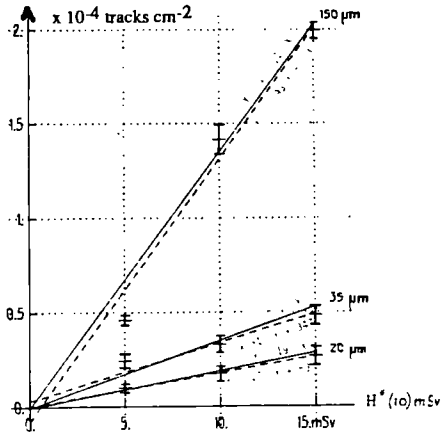


Fig. 1 : Dosemeter sensitivity (differential method) vs dose equivalent (3.3 MeV neutrons)

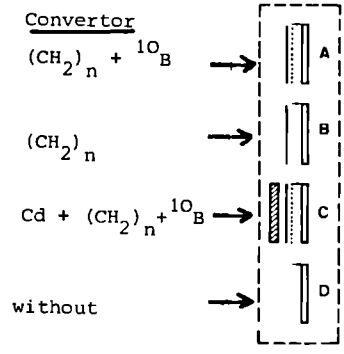


Fig. 2 : Dosemeter based on differential method

IV - Objectives for the next reporting period

Using the image analysis system our dosemeter will be calibrated in CADARACHE (SIGMA) and during the irradiations of CENDOS EURADOS;

An "electronic dosemeter" was tested in 1988 and our purpose is to compare the response of the two dosemeters built in our laboratory : the "DST dosemeter" and the "electronic one".

V - Other research group(s) collaborating actively on this project [name(s) and address(es)] :

Irradiations within the neutron joint irradiations of the CENDOS - EURADOS (n° 5)

Collaboration with the SIDR CEA (Fontenay-aux-Roses - CADARACHE)

VI - Publications

- Didier PAUL - DEA Physique Radiologique
  juin 1988 - TOULOUSE

- Results of background survey
  J.L. DECOSSAS, L. MAKOVICKA, D. PAUL, J.C. VAREILLE
  Report EURADOS CENDOS
  To be published

RADIATION PROTECTION PROGRAMME

Progress Report

1938

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Grenoble
85 X
F-38041 Grenoble Cédex

Contract no.: BI6-A-005-F

Head(s) of research team(s) [name(s) and address(es)]:

Mme S. Descours
Serv.de Prot.contre les Rayonn.
CEA, CEN de Grenoble
85 X
F-38041 Grenoble Cédex

Telephone number: 76-88.33.96

Title of the research contract:

Study of a transfer dosemeter for the determination of dose in tissue close to beta-radiation sources

List of projects:

1. Study of a transfer dosemeter for the determination of dose in tissue close to beta-radiation sources.

Title of the project
Study of a transfer dosemeter for the determination of dose in tissue close to beta radiation sources.

Head of project
Y. HERBAUT

Scientific staff
J.B. LEROUX, M. DELAHAIE.

I - Objectives of the project
- Evaluation of the uncertainties in beta absorbed doses measured with currently used extremity dosemeters.
- Use of an extrapolation chamber as a reference detector and TSEE or ultra thin TL dosemeters as transfer instruments.

II - Objectives of the reporting period
. Results obtained with an extrapolation chamber EIC1-FWT connected with a precise electronic chain.
. Study of the characteristics of TL or BeO dosemeters as transfer detectors.

III - Progress achieved

1/ Extrapolation chambers
Two extrapolation chambers FWT (Far West Technology) were used as reference detectors. Their entrance window is a graphite coated mylar foil, 0,83 $mg.cm^{-2}$ thick or a 7 $mg.cm^{-2}$ AI50 TE material absorber. Experiments with these two ionisation chambers irradiated by a $(Sr+Y)^{90}$ radioactive source of the Büchler facility have been carried out, their ratio giving the corresponding transmission factor T :

$T = 1,036$ ($\pm$ 1,8 %)

So we can reach the transmission ratio T' between a 7 $mg.cm^{-2}$ mylar entrance window and a 7 $mg.cm^{-2}$ ET one for the same radionuclide.

$T' = 0,98$ ($\pm$ 1,8 %)

The same measurements have been done with a Tl^{204} source associated to a filter according to the ISO recommendations. This source (Ø = 42 mm) was calibrated by LMRI.

$T = 0,93$ ($\pm$ 6,4 %)

$T' = 0,91$ ($\pm$ 6,4 %)

On the other hand, the computation of the area S of the collecting volume by the measurement of the capacity according to the J. BOHM's /1/ method has been carried out. This determination confirmed the results obtained, in electronic equilibrium conditions, with a Co^{60} calibrated beam.
We can compute also the minimum distance Yo between the two electrodes.
For the chamber with a 0,83 $mg.cm^{-2}$ entrance window :

$S = 0,866\ cm^2$ ($\pm$ 2,3 %)

Yo = 0,39 mm ($\pm$ 14 %)

For the chamber with a 7 $mg.cm^{-2}$ entrance window :

$S = 0,836\ cm^2$ ($\pm$ 1,9 %)

Yo = 0,35 mm ($\pm$ 11,7 %)

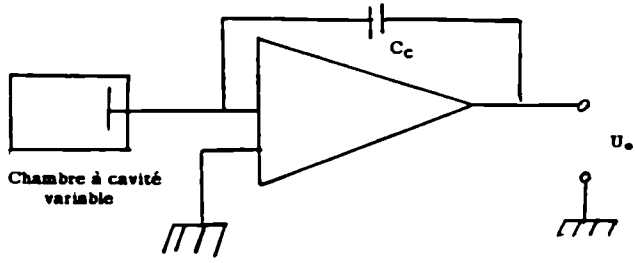
This method, described in the PTW-FREIBURG manual on extrapolation chamber according to Dr BOHM consists to measure the change Δuo, of the output voltage of the current measuring system, if we change the chamber voltage by Δu and if we call the feedback capacitor Cc (Cc = 101,21 pF ± 0,1 %).
So if C is the capacity of the extrapolation chamber

$$C = Cc \frac{\Delta uo}{\Delta u}$$

with $C = \varepsilon o \cdot S / (Y+Yo)$, so

$$Y = \frac{\varepsilon o \cdot S}{C} - Yo$$

a regression line through the data points $(\frac{1}{C}, Y)$ is used to compute the zero point Yo and the collecting area S.



These extrapolation chambers are associated to a precise electronic chain and a micro computer.
The uncertainties on temperature, pression, the feedback capacitor, the stopping power and the average energy per ion pair are taken into account.

2/ Irradiation facility
Moreover we can operate with a beta irradiation facility, built by CEA/FAR/DPT (France) and using large diameter beta sources calibrated by the primary laboratory (LMRI).

3/ Beta dosimetry using thermoluminescence and thermally stimulated exoelectron emission

TL dosemeters
TL dosemeters, each calibrated, are FLI thin layers from Teledyne. Their geometrical charactéristics are : 13 mm in diameter and a thickness of 130 µm. The calibration source is the Büchler Tl^{204} one with its own filter, as defined by ISO recommendations, giving a tissue dose rate of 165.5 $\mu Gy.h^{-1}$ at a 30 cm distance under a 7 $mg.cm^{-2}$ tissu depth.
Two measurements, for each dosemeter, are performed with the TOLEDO 654 reader :
. the first L_1 giving an information related to the irradiation.
. the second L_2 giving the noise of the sample.
So the difference L_1-L_2 is proportional to D_γ during the irradiation time.

The broad Tl^{204} source (Ø = 42 mm), calibrated by LMRI, at 30 cm, with its ISO filter exhibits.
. a dose rate equal to $3,91 . 10^{-2}$ Gy.h^{-1} (± 4 %) for the measurements performed with the FWT extrapolation chamber having a 7 mg.cm^{-2} entrance window.
. a $3,96 . 10^{-2}$ Gy.h^{-1} (± 6,2 %) beta dose rate for the FLi disks covered by a 7 mg.cm^{-2} A 150 film laying on a 20 mm perpex phantom.
There is good agreement between the two results. In each case, four experiments are carried out.

TSEE dosemeters
TSEE BeO thin film dosemeters, each calibrated, consist of a thin film about 100 nm thick on a graphite substrate of 1 mm. Detectors of 12 mm diameter have been used. They have been developed in recent years by the Battelle in Frankfurt and the University of Giessen (/2/). Because of the low stopping power of electron rays, a dosemeter must have a very small detection volume. The TSEE read out consists of a windowless methan gas flow multineedle counter. This device has been developed by M. PETEL (/3/). The high voltage applied is 2.35 kV, the flow gas rate 20 l.h^{-1}, the integration interval 200°C to 600°C and the main TSEE peak towards 350°C. The lowest integration limit was reached with a linear rate of 7°C. s^{-1} and the final temperature with a rate of 3°C.s^{-1}. Two measurements were done for each sample :
- a B_1 information in relation with the irradiation
- a B_2 information for the noise of the BeO dosemeter.
So the difference B_1-B_2 is proportional to the tissue dose under a fixed depth.
The BeO dosemeter, covered with a 7 mg.cm^{-2} equivalent tissue film and positioned on a 20 mm thick phantom, at a 30 cm distance from the source, was irradiated successively by the PTB TL^{204} point source and the LMRI Tl^{204} broad source (Ø = 42 mm). For five experiments performed, we obtained in this last case $D_7^0 = 4.72 . 10^{-2}$ Gy.h^{-1} (± 7,5 %) instead of $3,91.10^{-2}$ Gy.h^{-1} for the FWT extrapolation chamber.
So we have tried to study such dosemeters from a systematic point of view. 30 BeO dosemeters have been bought to the firm "Staatliches Materialprüfungsamt NRW" of Dortmund (W. Germany). They are irradiated by a calibrated Co^{60} source, covered with a 7 mg.cm^{-2} film under electronic equilibrium : 5.2 mm of perpex before and behind the dosemeter.

Uniformity of the set : we have obtained for the mean response R
$$R = 6,057 . 10^6 \text{ digits} / \text{Gy} \ (\pm 125\ \%)$$

Long term stability : for the same dosemeter, we have performed 6 measurements at different moments : t = 0,1 day, 2d, 9d, 14d, 28 days and obtained for the mean :
$$R = 9,39 . 10^6 \text{ digits} / \text{Gy} \ (\pm 48\ \%)$$
A counter calibration is performed with a C^{14} source at the beginning and the end of eachday.

Short term stability : A BeO dosemeter was irradiated by Co^{60} 7 times and read immediately in the same day. This set was repeated 4 times ; we have obtained for the mean R :
$$R = 6,091 . 10^6 \text{ digits} / \text{Gy} \ (\pm 11\ \%)$$
(the mean accuracy is equal to the product of the standard deviation by a factor of 3, as recommended by LMRI).

Because of these great in accuracies, it seems to us that these BeO dosemeters are unusable actually.

IV - Objectives for the next reporting period

The future research would be split as follows :

. Study of dosimetric characteristics, for beta irradiation, in a closeness geometry, of an ultra. Thin dosemeter, thermoluminescent or based on the thermally electron emission, and comparison with the results given by an extrapolation chamber.

. Comparison of the results given by a point source (PTB) and a broad circular source (Ø = 42 mm LMRI) for two radionuclides - $^{90}Sr+^{90}Y$ and ^{204}Tl.

V - Other research group

VI - References

/1/ Instruction manual : extrapolation chamber for the measurement of the absorbed dose rate to tissue for beta radiation, according to Dr BOHM, PTW Freiburg (W Germany)

/2/ C.U. Wieters et al : Electron dosimetry with thermally stimulated exoelectron emission (TSEE) - Phys. Med. Biol. 1984 - Vol. 29 n°9 (1097-1107)

/3/ M. PETEL et al : Multineedle counter with cathodic focusing (MNCF) used for TSEE dosimetry. RPD - Vol n°314 (171-173).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Kernforschungsanlage Jülich GmbH
Postfach 1913
D-5170 Jülich 1

Contract no.: BI6-A-007-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof.Dr. L.E. Feinendegen
Institut für Medizin
Kernforschungsanlage Jülich
Postfach 1913
D-5170 Jülich 1

Telephone number: 2461-61.64.43

Title of the research contract:

Application of microdosimetric methods to radiation protection.

List of projects:

1. Implementation of a low-pressure proportional counter for use as a diagnostic working area and environmental dosemeter of high sensitivity and high dynamic range in LET.
2. Magnitude and meaning of local dose profiles around single decays of incorporated radionuclides in radiation protection.

Title of the project no.: 1

Implementation of a low-pressure proportional counter for use as a diagnostic working area and enviromental dosemeter of high sensitivity and high dynamic range in LET

Head(s) of project:

L.E. Feinendegen

Scientific staff:

L.E. Feinendegen, J.Booz, Th. Schmitz

K. Morstin (Univ. Krakow), A. Dydejczyk (Univ. Krakau)

I. Objectives of the project:

- Adaption of the KFA counter to practical requirements
- Developement of a simple external calibration method for the KFA counter
- Collection of information on dose equivalent distributions of neutron/gamma fields at working areas with the KFA counter

II. Objectives for the reporting period:

- Participation in the final interpretation of the intercomparison 1986 and 1987 at the PTB in Braunschweig
- Experimental determination of the photon response of the KFA counter
- Determination of the Townsend coefficient as a function of the reduced field strength for a ½" Rossi type proportional counter
- Measurement of dose equivalent in and around an experimental bunker at the heavy-ion accelerator of the Gesellschaft für Schwerionenforschung (GSI), Darmstadt

III. Progress achieved:

A diagnostic working area monitor called the KFA counter, has been designed and constructed. Its response, optimized to match $H^*(10)$ for neutrons between thermal and 20 MeV, and its nonlinear amplifying system, in conjunction with a data acquisition system, enables the measurement of microdosimetric distributions and the evaluation of absorbed dose, dose equivalent, dose and dose-equivalent rate for neutrons and gammas in one single reading. Details of the developement and first results of performance tests together with related references have been specified in the final report BIO-A-288-D, 1980-1984 and in the progress reports BI6-007-D, 1985, 1986 and 1987 to the Commission.

Participation in Intercomparisons at the PTB Braunschweig

During two intercomparison campains at the accelerator and Reaktor facilities of the PTB in Braunschweig in 1986 and 1987 measurement were performed in monoenergetic neutron beams of energies between thermal and 14.8 MeV, as well as in the fields of a ^{60}Co standard source and a heavy water moderated ^{252}Cf source.

The objective of the intercomparisons was to identify those parameters, which influence the neutron energy dependence of the detectors responses and to work out recommendations for further improvements. A detailed report on the first part of the intercomparion in 1986 was sent to the CEC in 1988 and is available as preprint. The final report is in preparation and will be published in the journal 'Radiation Protection Dosimetry' as part of the proceedings of the workshop on the 'Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection' in 1989.

Experimental Determination of the Photon Response of the KFA Counter

In order to determine the photon response of the KFA counter, experiments were performed in well defined, almost monoenergetic photon fields between 10 keV and 1.25 MeV at the PTB in Braunschweig. In each radiation field, wall thicknesses of 4 mm, 15 mm and 20 mm tissue equivalent material (polyethylene and A-150) were were used. In addition calculations, which simulated the experiments, were perfomed in order to guide the interpretation of the measured dose distributions.

The agreement between calculated and measured dose distributions was very good. The calculation showed the contribution of photoelectrons and compton electrons, as well as the contributions of stoppers, crosser and starters to the dose absorbed in the sensitive volume of the counter.

Figure 1 summarizes the results of the experiments in terms of the dose equivalent response with respect to ambient dose equivalent, $H^*(10)$, as a function of incident photon energy for a wall thickness of 4 mm, 15 mm and 20 mm. For incident photon energies above 30 keV, the reponse is always better than 0.85. The peak at

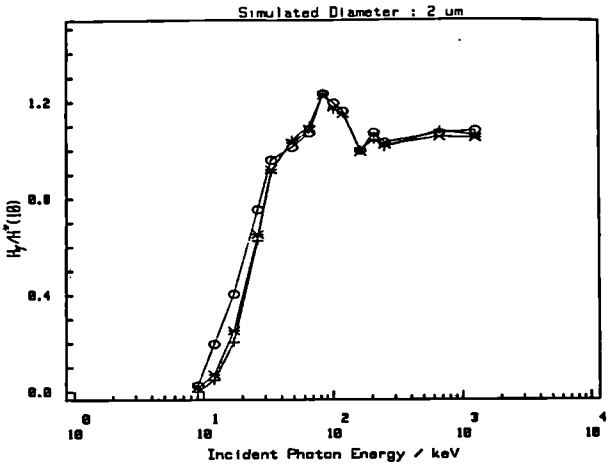


Figure 1 : Measured dose equivalent response, $H_m/H^*(10)$, for the KFA counter with 4 mm (circles), 15 mm (stars) and 20 mm (crosses) wall thickness as a function of incident photon energy.

100 keV ($H_m/H^*(10)$ = 1.23) is probably due to the contribution of stoppers and insiders to the measured absorbed dose. The decrease of the response below 26 keV is due to an underestimation of the LET of the secondary electrons. In addition, the reponse is decreased because both A-150 plastic and the filling gas are no longer tissue equivalent below 40 keV photon energy. For example, the ratios of the mass energy-transfer coefficients for 10 keV photons are 0.75 and 0.66 for respectively A-150 and methane-based tissue equivalent gas, relative to ICRU muscle tissue.

Determination of the Townsend Coefficient in a ½" Rossi Counter

For this work the behavior of the gas-gain was investigated in a ½" spherical TEPC with helix. The counter was filled with methane based tissue equivalent gas. The internal ^{244}Cm alpha source was used for all measurements. The gas gain was determined for six different gas pressures between 56.27 Torr and 562.69 Torr, which corresponds to simulated diameters between 1 μm and 10 μm.

The important parameter governing the gas amplification is the number of electrons created per unit length, which is called the Towsend coefficient, α_T. The Townsend coefficient was determined from experiments as described above. Results show that under these conditions α_T rises linearly with the reduced field strength over a certain range. Above this range α_T first rises less steeply, reaches a maximum and then decreases with increasing field strength. Possible explanations found for the formation of the maximum and the consecutive decrease are as follows : At pressures above 168.8 Torr, a space charge effect and the loss of electrons at the helix; at pressures below 168.8 Torr, an energy effect, i.e., the electrons gain so much energy that the probability of ionizing collisions decreases.

IV. Objectives for the next reporting period:

- Further improvement of the dose-equivalent response of the KFA counter

- Collection of information with the KFA counter on dose-equivalent distributions of neutron/gamma fields at simulated realistic radiation protection fields and at working areas, e.g. at Cadarache and the accelerator and reactor facilities of the Crakow University

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Members of EURADOS Committee I on "Dose equivalent meters based on microdosimertic techniques"

- G. Portal, CEA Fontenay-aux-Roses

- G. Dietze, H.M. Kramer, PTB Braunschweig

VI. Publications:

Morstin, K., Dydejczyk, A., Booz, J.
High Energy Neutron Interactions with Tissues and Tissue Substitutes
Nuclear and Atomic Data for Radiotherapy and Related Radiobiology,, S.239-262 (IAEA, Wien, 1987).

Dietze, G., Edwards, A.A., Guldbakke, S, Kluge, H., Leroux, J.B., Lindborg, L., Menzel, H.G., Nguyen, V.D., Schmitz, Th., Schumacher, H.
Investigation of Radiation Protection Instruments Based on Tissue-Equivalent Proportional Counters. Results of an Intercomparison
Commission of the European Communities, EUR (Luxembourg:CEC) 1988.

Morstin, K., Dydejczyk, A., Booz, J.
Nuclear Model Calculations for High Energy Neutron Dosimetry
Radiat. Prot. Dosim. 23, No.1/4 pp. 35-39 (1988)

Schmitz, Th., Morstin, K., Booz, J.
Performance of a Dose Equivalent Meter for Area Monitoring
Radiat. Prot. Dosim. 23, No.1/4 pp. 235-238 (1988)

Bednarek, B., Olko, P., Booz, J.
Double Peak Effect in Microdosimetric Proportional Counters and it's Interpretation
Nucl. Instr. Meth. Phys. Res. A274, pp. 349-358 (1989).

Booz, J., Morstin, K., Schmitz, Th.
Direct Experimental Assessment of H_{eff} with Tissue Equivalent Proportional Counters Questions its Calibration in Terms of $H^*(10)$
Seminar on Implementation of Dose Equivalent Operational Quantities into Radiation Protection, Braunschweig, 7-9 Juni 1988.

Booz, J.
Microdosimetric Applications in Radiation Biology
7^{th} Annual Meeting of the European Society for Therapeutic Radiology and Oncology, ESTRO, Den Haag, 2-8 September 1988.

Booz, J., Olko, P., Schmitz, Th., Feinendegen, L.E., Morstin, K.
The KFA Counter - Its Photon and Neutron Responses, Sensitivity, Performance, and its Potential for Future Developement
Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection
Schloß Elmau, 18-20 Oktober 1988

Schmitz, Th., Booz, J.
Measurements of the Gas-Amplification Coefficient in a TEPC
Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection
Schloß Elmau, 18-20 Oktober 1988

Schmitz, Th., Kramer, H.M., Booz, J.
Assessment of the Photon Response of a TEPC as a Contribution to Implementing Operational Quantities for Dose Equivalent in Radiation Protection
Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection
Schloß Elmau, 18-20 Oktober 1988

Menzel, H.G., Lindborg, L., Schmitz, Th., Schuhmacher, H., Waker, A.J.
Intercomparison of Dose Equivalent Meters Based on Microdosimetric Techniques : Detailed Analyses and Conclusions
Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection
Schloß Elmau, 18-20 Oktober 1988

Title of the project no.: 2

Magnitude and meaning of local dose profiles around single decays of incorporated radionuclides in radiation protection

Head(s) of project:

L.E. Feinendegen

Scientific staff:

L.E. Feinendegen, J.Booz, P. Olko

I. Objectives of the project:

- Assesment of energy deposition distributions and local dose profiles around single decays of incorporated radionuclides and other radiation sources
- Implications for radiation protection limits of incorporated radionuclides
- Understanding of the underlying radiation mechanisms

II. Objectives for the reporting period:

- Development of analytical functions describing energy-deposition distributions from protons and alpha particles
- Calculations of local dose profiles around charged particles tracks
- Development of methods for the evaluation of biological response functions

III. Progress achieved:

Energy Deposition Distributions for Heavy Ions

In the last years report we had shown that energy deposition spectra induced by protons and alpha particles in such small sites as a DNA helix or nucleosomes can be described with analytical functions. This analytical recipe distinguishes between events produced directly by ions crossing a sensitive site (ion events) and by those passing outside the site and depositing energy only by delta-electrons. Straggling of energy deposited by ion events was expressed by a 2 parameter Fermi-like function. Delta-electrons action was approximated by an exponential function of a mean number of ionization produced in the site by efflux of secondary electrons.

These calculations were further continued in order to generalize the model for different ion energies (0.3-10 MeV/amu), site diameters (1-1000nm) and types of ion (from protons to oxygen ions). Microdosimetric distributions were evaluated from a set of tracks simulated by an improved version of the track structure code MOCA-14. Simulation of tracks for ions with $Z > 1$ was based on the assumption that the energetic spectrum of secondary electrons in water vapor is similar for different ions of the same velocity. The mean free path between ionizing collisions for a given ion was evaluated from that for protons using effective charge as a scaling parameter. Model parameters were fitted to calculated distributions using a nonlinear least-squares method (NL2SOL program from ZAM-KFA mathematical library). This same set of tracks was used to calculate radial dose distributions around an ion path. The calculated radial dose distributions are in good agreement with a broad set of experimental data (Fig.1).

Fig.2. shows frequency distributions of ionizations produced by 0.3 MeV/amu alpha particles in spheres of diameters between 5 and 1000 nm. With decreasing site diameter, a fraction of indirect events increases. These are events due to delta-electrons ejected to site by alpha particles passing outside the site. The figure demonstrates that the distribution for small site sizes are very different from the "classical" triangular distributions.

These realistic microdosimetric distributions for sites of nanometer diameters were used to deconvolute biological response functions for DNA double strand breaks. A good correlation between these functions and response functions for cell inactivations was obtained.

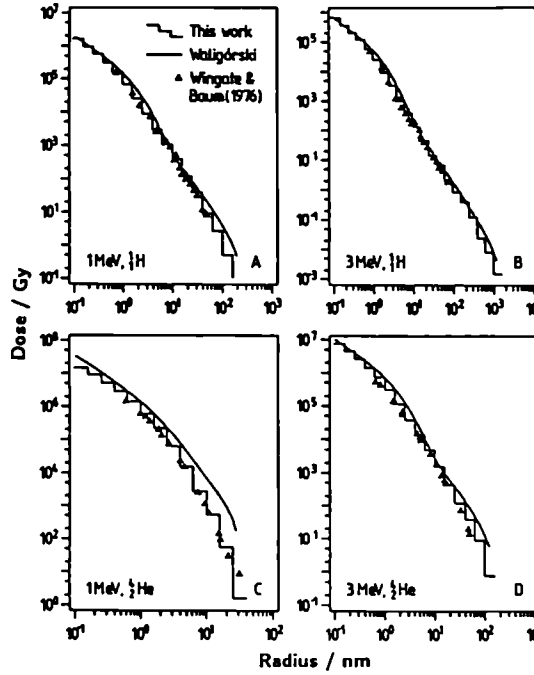


Fig. 1.
Comparison of Monte-Carlo calculations of radial dose distributions (MOCA-14; histograms) with model calculations of (Waligorski et. al., 1986; lines) and measurements of (Wingate & Baum, 1976; triangles)

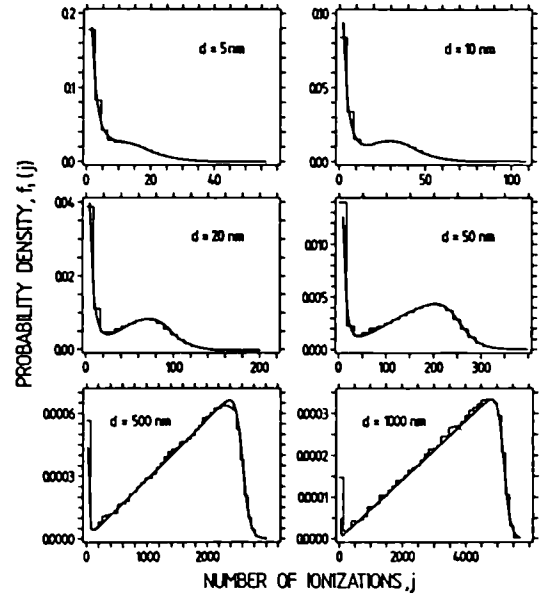


Fig.2.
Frequency distribution of ionizations produced by 0.3 MeV/amu alpha particles in spheres of diameters from 5 to 1000 nm. Histograms denote Monte-Carlo calculations, bold lines - model.

IV. Objectives for the next reporting period:

- Application of the developed analytical functions for the description of ionization distributions from charged particles to various radiation modalities.

- Further development of the methodology for the evaluation of biological response functions

- Quantification of radiation quality with the help of biological response functions.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- H. G. Paretzke, GSF Neuherberg

- D.T. Goodhead, MRC Harwell

VI. Publications:

Olko, P., Booz, J., Paretzke, H., Wilson, W.E.
Energy Deposition in Nanometer Sites Based on Track Structure Calculations
Proceedings of the IAEA Group Meeting on Atomic and Molecular Data for Radiotherapy, Wien, (in press).

Booz, J., Feinendegen, L.E.
A Microdosimetric Understanding of Low-Dose Radiation Effects
Int. J. Radiat. Biol. 53, S. 13-21 (1988)

Bond, V.P., Feinendegen, L.E., Booz, J.
What is a "Low Dose" of Radiation ?
Int. J. Radiat. Biol. 53, S. 1-12 (1988)

Feinendegen, L.E., Bond, V.P., Booz, J., Mühlensiepen, H.
Biochemical and Cellular Mechanisms of Low-Dose Effects
Int. J. Radiat. Biol. 53, S. 23-37 (1988)

Feinendegen, L.E., Booz, J., Bond. V.P.

Empirical Approaches to Developement of Biophysical Models; Biophysical Aspects

XXI Radiobiological and Chemical Physics Contractor's Meeting, Los Alamos, 11-12 Mai 1988.

Booz, J., Feinendegen, L.E., Olko, P., Bond, V.P.

Empirical Approaches to Developement of Biophysical Models; Differentiation of Cellular Responses to Specific and Non-Specific Effects

XXI Radiobiological and Chemical Physics Contractor's Meeting, Los Alamos, 11-12 Mai 1988.

Olko, P., Booz, J., Paretzke, H., Wilson, W.E.

Energy Deposition in the Nanometer Sites Based on the Track Structure Calculations

IAEA group meeting on atomic and molecular data for radiotherapy, Wien, 13-16 Juni 1988

Olko, P., Schmitz, Th., Morstin, K., Dydejczyk, A. and Booz, J.

Microdosimetric Distributions for Monoenergetic Photon Fields

Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection

Schloß Elmau, 18-20 Oktober 1988

Olko, P., Schmitz, Th., Booz, J.

Energy Deposition and Ionization Yields of Photon Radiation in Sites of DNA Dimensions

21st annual meeting of European Soc. of Radiation Biology, Tel Aviv, 24-30 October 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-A-232-E

Universidad Autonoma de Barcelona
Serv. de Fisica de las Radiaciones
E-08193 Bellaterra, Barcelona

Head(s) of research team(s) [name(s) and address(es)]:

Prof. F. Fernandez Moreno
Departamento de Fisica
Univ. Autonoma de Barcelona
E-08193 Bellaterra, Barcelona

Telephone number: 3/6920200 1659

Title of the research contract:

Heating by laser of thermoluminescence dosimeters; application to the measurement of low energy beta rays.

List of projects:

1. Heating by laser of thermoluminescence dosimeters, application to the measurement of low energy beta rays.

TITLE OF THE PROJECT Nº: B-16-A-232-E

CHAUFFAGE PAR LASER DE DOSIMETRES THERMOLUMINESCENTS: APPLICATION A LA MESURE DES BETAS DE FAIBLE ENERGIE

HEAD OF PROJECT:

Profesor F. Fernández Moreno
Departamento de Física.
Universidad Autónoma de Barcelona.
E- 08193 Bellaterra, Barcelona.

SCIENTIFIC STAFF:

F. Fernández Moreno
A. Vidal Quadras
C. Baixeras

I. OBJECTIVES OF THE PROJECT:

Application to beta dosimetry of laser heating in thermoluminiscence dosimeters.

II. OBJECTIVES FOR THE REPORTING PERIOD:

During the first stage of the study we have carried out the following points:

- Manufacturing of our own $CaSO_4$:Dy
- Study of the $CaSO_4$:Dy and CaF_2:Mn characteristic thermoluminiscence parameters.
- Setting up of a VALLADAS thermoluminiscence reading system.
- Performance of the first tests of the manufactured dosimeters, using a $Sr\text{-}Y^{90}$ source.

III. PROGRESS ACHIEVED:

1. Methodology:

The memory phenomenon in thermoluminiscence materials, exposed to ionizing radiation, is well know. Reading of the recorded information is carried out by means of a controlled heating, in order to register the light emission from the dosimeter. This heating may be out by several methods. We have used in this work the laser heating as well as a comparison with conventional heating. For this purpose, we have a conventional thermoluminiscence reader (TL), toghether with a software package, that have been set up in this work.

2. Results:

2.1. TL Reading system calibration: We have carried out a complete calibration of all thermal parameters with the aim of optimizing the usage of the Tl reader. At the same time, the $Sr\text{-}Y^{90}$ source has been calibrated in order to obtain an effective irradiation device, able to irradiate dosimeters to well known doses.

2.2. Software development: In order to completely optimize the TL reader, it has been necessary to develop two program packages. One of them is completly dedicated to collectivy data from the TL reader system and its containe the TLGRAF and TLGROW programs. The other one is dedicated to the data treatment and contains a program package called PAK.

2.3. Basic study of $CaSO_4$:Dy This study has been carried out with powder manufactured by ourselves. We have obtained the typical thermoluminiscence parameters for this material.

2.4. Basic study of CaF_2:Mn This study is of the same characteristic that the one in 2.3 an it is being completed at present.

3. Discussion:

During this first step we have been working in the setting up of our conventional TL reader, with the aim of having a completely operational and reliable instrumentation, in order to carry out deteiled studies on every thermoluminiscent material that will be used in the project, and to analize the reponse of these materials to different kinds of radiation and different doses.

As this instrument is conceived, a great flexibility is expected, in particular in front of a laser heating device, less versatile as many relevant TL thermal parameters cannot be contolled with the same efficiency than in a conventional system. It is, then, of great interest to have both TL reading systems for developing new beta dosimeters, as both instruments are complementary within this project.

A first test in the conventional TL reading system has been carried out by measuring $CaSO_4$:Dy, well known in the field of dosimetry, and the results obtained agree with those published by other authors.

IV. OBJECTIVES FOR THE NEXT REPORTING PERIOD:

The next step in our work will be the study of the reponse of our dosimeters as a function of the energy of the recorded beta particles. With this purpose, we have a $Sr-Y^{90}$ source, with filters that allow the selection of the radiation energy. This study will be completed with an analysis of the response as a function of the powder grain size and of the thickness of the material deposited on the dosimeter.

In addition, some test with conventional system will be carried out to guarantee reproductibility and quality in all sets of thermoluminiscent material manufactured in our laboratories.

V. OTHER RESEARCH GROUPS COLLABORATING ACTIVELY ON THIS PROJECT:

Centre d'Electronique de Montpellier,

Université des Sciences et Techniques du Languedoc,

Place Eugene Bataillon,F-34 060 Montpellier Cedex,

Professeur Jean Gasiot.

CEA - CEN/STEP-STID, BP 6, F-92 260 Fontenay-aux Roses,

Dr. G. Portal.

VI. PUBLICATIONS:

In preparation.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université des Sciences et
Techniques du Longuedoc (USTL)
U.S.T.L.
Place Eugène Bataillon
F-34060 Montpellier Cédex

Contract no.: BI6-A-231-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J. Gasiot
Ctre. d'Electr.de Montpellier
U.S.T.L.
Place Eugène Bataillon
F-34060 Montpellier

Telephone number: 67/52 56 33

Title of the research contract:

Heating by laser of thermoluminescence dosimeters; application to the measurement of low energy beta rays.

List of projects:

1. Heating by laser of thermoluminescence dosimeters; application to the measurement of low energy beta rays.

Title of the project n° **CONVENTION N° B 16* 231-F (EDB)**

**CHAUFFAGE PAR LASER
DE DOSIMETRES THERMOLUMINESCENTS.
APPLICATION A LA MESURE DES BETAS DE FAIBLE ENERGIE.**

Head of project:

Professeur Jean Gasiot,
Equipe Dosimétrie,
Centre d'Electronique de Montpellier,
Université des Sciences et Techniques du Languedoc, Case 083,
Place Eugène Bataillon, F-34 060 Montpellier Cedex.

Scientific staff:
Jean Gasiot
Jean-Pierre Charles

I. Objectives of the project

Application à la dosimétrie béta du chauffage de dosimètres thermoluminescents par laser.

II. Objectives for the reporting period

Dans la première phase de cette étude nous avons:

- préparé des poudres thermoluminescentes de $CaSO_4$.
- analysé la pénétration du rayonnement béta dans quelques matériaux qui entrent dans la réalisation des dosimètres les plus utilisés.
- préparé de nouveaux dosimètres utilisant des matériaux thermoluminescents couramment utilisés: CaF_2, $CaSO_4$, LiF, $Li_2B_4O_7$....
- réalisé les premiers tests sur des dosimètres thermoluminescents préparés spécialement pour ce programme, ont été irradiés en utilisant l'émission de sources $Sr\text{-}Y^{90}$.

III. Progress achieved

1. Methodology

Lors de l'exposition d'un dosimètre thermoluminescent à une radiation ionisante, il se produit une série de transitions électroniques entraînant le stockage d'une information. Sa lecture s'effectue lors du réchauffement des dosimètres qui donne lieu à une émission lumineuse - thermoluminescence - proportionnelle à la dose. Le chauffage par Laser présente des avantages considérables par rapport aux techniques classiques, il est plus rapide et donne lieu à une émission plus intense. L'intensité du signal lumineux est à peu près proportionnelle à la vitesse de chauffage; pour un temps de 10 ms le gain en intensité est de l'ordre de 1000 par rapport à un chauffage conventionnel qui nécessite un temps de l'ordre de 10 secondes. La rapidité de chauffage nécessite cependant des dosimètres fins, qui sont les seuls à pouvoir être chauffés rapidement sans gradiant de température préjudiciable à la qualité de la lecture de doses. Les capteurs fins étant particulièrement bien adaptés à la dosimétrie des radiations peu pénétrantes, la technique de chauffage par Laser semble toute indiquée. La mesure des doses bétas de faible énergie peut s'effectuer dans de meilleures conditions. Cette observation est à la base de notre étude, elle l'a motivée.

2. Results

2.1. Présentation des matériaux et des supports : Nous avons, dans un premier temps, sélectionné le $CaSO_4$ qui présente pour cette étude de nombreux avantages (absorption importante à 10 microns, particulièrement bien adaptée au chauffage par Laser). Nous avons préparé des dosimètres constitués par des dépôts de poudres thermoluminescentes sur divers supports; principalement lames de verre et **Kapton**. L'utilisation de ce type de support est intéressante en dosimétrie **Béta** où l'on a souvent besoin de capteurs souples.

2.2 Préparation des matériaux. Nous partons de sulfate de calcium amorphe et de son dopant mixés dans un bain d'acide sulfurique fumant qui est chauffé pour obtenir la dissolution totale. Par distillation de l'acide, nous obtenons des poudres monocrystallines qui après séchage et recuit à 900 degrés Celsius sont broyées et tamisées avant d'être utilisées pour la préparation des dépôts.

2.3. Fabrication du dosimètre. Nous avons retenu la **Sérigraphie** pour le couchage des matériaux thermoluminescents ($CaSO_4$; LiF; CaF_2...) sur leur support. Les tests d'homogénéité et de reproductibilité effectués

sur plusieurs lames montrent un écart-type en général inférieur à 5%. Les résultats sur la linéarité de la réponse avec la dose reçue et le fading sont en accord avec ceux généralement obtenus sur ce type de dosimètre.

2.4. Présentation du lecteur laser. Un faisceau Laser CO_2 stabilisé à 10W environ, d'un diamètre compris entre 300µm et 2mm, permet le chauffage des dosimètres. Un micro-ordinateur assure la commande, la gestion et le traitement de toutes les données recueillies lors des lectures.

2.5. Résultats de mesures d'exposition à un rayonnement béta. Nous avons effectué toute une série d'irradiations pour des doses comprises entre 0,2 et 2 cGy. SrY90 sur des LiF(6 et 7) , $CaSO_4$(Mn/Dy), CaF_2:Mn, Al_2O_3. Seuls pour l'instant les résultats sur $CaSO_4$(Mn/Dy) sont très probants. Nous sommes en train de préparer des mesures sur des plaques de grande taille (22 x 22 x 0,01 cm^3) qui présentent un intérêt certain pour la cartographie des faisceaux et des sources.

3. Discussion

Cette première partie de notre étude a été consacrée à la mise en place des divers éléments, poudres, lecteurs et plaques luminescentes qui nous permettront de délimiter l'intérêt de la technique de chauffage Laser à la dosimétrie béta. Les points importants ont été la préparation de sulfates et de dosimètres, nous avons aussi dû améliorer notre lecteur et le traitement des données. La préparation au laboratoire de matériaux luminescents nous a conduit à vérifier leurs caractéristiques afin de les homologuer. Ce point est primordial dans la mesure où, d'une préparation à l'autre, nous avons pu, suivant la provenance des ingrédiants, observer des différences considérables dans les résultats. Nous avons calculé suivant les procédures habituelles le pouvoir d'arrêt par ionisation et par radiation sur des matériaux standards et sur les quelques matériaux utilisés habituellement en dosimétrie par thermoluminescence. Nous avons enfin effectué les premières mesures de thermoluminescence sur quelques dosimètres irradiés béta, ceci nous permet de connaître les ordres de grandeur des signaux. L'optimisation de nos préparations et de nos lectures, doit nous permettre d'améliorer ces résultats. Cette étude permettra de mieux cerner la réponse des dosimètres thermoluminescents fabriqués à Montpellier.

IV. Objectives for the next reporting period

La prochaine phase de notre travail sera axée sur la réponse de nos dosimètres optimisés en fonction de l'énergie des béta utilisés lors de l'irradiation. Ne disposant pas de sources monoénergétiques, nous effectuerons les irradiations en interposant des filtres entre les sources et les dosimètres: ce type d'expérience devrait nous permettre d'évaluer grossièrement les spectres en energie des bétas utilisés pour l'irradiation. Nous projetons de déterminer l'intérêt de nouveaux dosimètres, CaF_2:Mn sur AsGa, sélectionnés pour leur épaisseur submicronique, et de nouvelles plaques thermoluminescentes (20 x 20 cm^2) de grande taille de technologie standard utilisables pour la détermination de la topographie de l'irradiation.

V. Other research groups collaborating actively on this project:

Laboratorio de Fisica de las Radiaciones, Dept. de Fisica, Universitad Autonoma de Barcelona, Bellaterra (Barcelona), Pr. F. Fernandez

CEA - CEN/STEP-STID, BP 6, F-92 260 Fontenay-aux-Roses, Dr. G. Portal

CEA - Service de Protection contre les Rayonnements, SPR/GMI, CEG, F-38 041 Grenoble CEDEX,
Dr. Y. Herbaut

VI. Publications:

Pour l'aspect dosimétrie béta, voir le rapport joint.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-A-019-UK

United Kingdom Atomic Energy
Authority, UKAEA
11 Charles II Street
GB London, SW1Y 4QP

Head(s) of research team(s) [name(s) and address(es)]:

Mr. J.A.B. Gibson
Environmental & Med.Sc.Div.
Harwell Laboratory of the UKAEA
Didcot
GB Oxon OX11 ORA

Telephone number: 0235-24141/4075

Title of the research contract:

Gamma-ray dosimetry, neutron dosimetry and spectrometry.

List of projects:

1. Factors affecting thermoluminescent output in LiF (TLD 600 and TLD 700) using glow curve analysis.
2. Neutron spectrometry.
3. Development of a CR-39 personnel neutron dosimeter.

Title of the project no. 1

Factors affecting Thermoluminescent Output in LiF (TLD-600 and TLD-700) using Glow Curve Analysis

Head(s) of project:

M Marshall

Scientific staff:

J A Douglas
C A Perks
R K Bull
J A B Gibson

I. Objectives of the project:

To provide a more general understanding of thermoluminescence by studying the effect on glow curve structure of various factors such as type of radiation, annealing conditions and temperature during irradiation. Particular areas to be studied include: the effect of cooling rate during anneal on peak positions; the effect of doses in the supralinear region on glow curve shape; UV phototransfer; and UV sensitisation. The study depends on appropriate analysis of glow curves into their individual glow peaks.

II. Objectives for the reporting period:

The glow-curve fitting and analysis routines will be used to analyse experimental data previously obtained. Additional experiments to complete and supplement sets of data will be performed as required. The techniques developed and results obtained will be written up for publication. The theoretical study of TL fading will be completed.

1. Introduction

Thermoluminescent dosemeters (TLDs) are widely used as personal dosemeters (both for whole body and extremity monitoring) in the nuclear industry and for those outside the nuclear industry exposed to ionising radiation. They have also found applications in determining doses to patients undergoing radiotherapy and, in nuclear engineering, to measure high doses in hostile environments.

In thermoluminescent (TL) materials, defects in the lattice structure create trapping centres in the forbidden band between the full valence band and empty conduction band. During irradiation, electrons (and holes) are excited into these traps. The trapped electrons (holes) are stored until they are released by heating the material and there is a high probability that the released electrons and holes will recombine emitting photons of light. This is the thermoluminescent signal. Increasing the temperature of the TL material gives rise to a glow curve, the variation of light output with time. This consists of a series of overlapping TL peaks, each peak associated with a particular trap depth.

Despite their widespread use, the detailed physical principles of thermoluminescent behaviour are not fully understood. This project was established to gain a more general understanding of thermoluminescence by developing techniques for glow curve analysis and applying them to determine the effect on individual glow peaks as a function of the previous treatment of the TL material. The mathematical formalism of TL emission cannot be solved analytically. Therefore, modern numerical techniques have been applied to theoretically model glow curve characteristics. Finally the expertise developed at Harwell into thermoluminescent behaviour has been applied to solve practical problems arising in thermoluminesence dosimetry.

2. Glow Curve Analysis

For linear rise in temperature with time, the glow curve from an irradiated TLD shows several, superimposed, characteristic glow peaks. To understand the behaviour of individual glow peaks it is necessary to resolve them from the glow curve. Three techniques have been developed at the Harwell Laboratory: non-linear least squares fitting; division of glow curves by a reference glow curve; and glow peak subtraction. The computer programs written to implement these techniques can all be run on an IBM compatible personal computer. These techniques are being used routinely to analyse glow-peak behaviour for TLDs exposed to a variety of conditions and results for the dose-response relationship of individual peaks for TLD-700 thermoluminescent ^{7}LiF, from the Harshaw Chemical Co, irradiated by gamma-rays in the dose-range 10 mGy to 20 kGy are presented.

2.1 Non-linear least squares fitting

If the shape of the individual glow peaks can be described mathematically, with appropriate variable parameters to define their position, height and width, then the experimental glow curves can be fitted by a set of individual glow peaks. A non-linear, least squares fitting, method based on a general purpose fitting programme called FATAL[1] has been developed. Initially this used the first order kinetic equation derived by Randall and Wilkins[2] for a constant heating rate to fit glow curves from TLD-700 ^{7}LiF:

$$I(T) = n_o.s.\exp(-E/kT).\exp\left[-\frac{s}{\beta}\int_{T_o}^{T}\exp(-E/kT)\,dT\right]$$

where: n_o is the number of trapped electrons at temperature T_o
s is the frequency factor (s^{-1})
E is the trap depth (eV)
T is the temperature (Kelvin)
k is Boltzmann's constant (eV K^{-1})
and β is the heating rate (s^{-1}).

Unfortunately $\int_{T_o}^{T}\exp(-E/kT)\,dT$ is not analytically soluble, and so this integral has to be solved numerically many times for each glow peak and for many iterations. Hence fitting the glow peaks using this equation is a lengthy process. Nevertheless, fitting the glow curves of irradiated ^{7}LiF in this way showed that 1st-order kinetics is a sufficient approximation.

An alternative approximation to the shape of 1st-order glow curves, which is much more rapidly evaluated, is that derived by Podgorsak et al[3]:

$$I = I_m \exp\left[1+\left(\frac{T-T_m}{T_m}\right) - \exp\left[\frac{(T-T_m)E}{kT_m^2}\right]\right]$$

where T is the temperature (K)
E is the trap depth (eV)
k is Boltzmann's constant (eV K^{-1})
I_m is the maximum intensity of the peak
T_m is the temperature at which the maximum intensity occurs (K).

This method has been used to analyse glow curves routinely. However, for very complex glow curves where there are many closely overlapping peaks of similar intensity (eg for highly irradiated TLDs of LiF), it is difficult, using glow-curve fitting techniques, to resolve all the peaks efficiently and accurately.

2.2 Glow curve division

To supplement the non-linear least squares method in resolving very complex glow curves, a new technique for analysis of glow curve structure has been developed. This compares experimental glow curves with a 'standard' glow curve which is well characterised. The experimental and standard glow curves are first normalised to unit dose. Then the ratio of the TL intensity at each temperature along the experimental glow curve to the TL intensity at corresponding temperatures on the 'standard' glow curve is determined. The ratio curve so obtained has flat regions where individual glow peaks dominate. The value of the ratio in these regions gives the relative sensitivity for that glow peak relative to the same peak on the standard curve.

2.3 Glow curve subtraction

Given the relative sensitivities of individual peaks from the glow curve division technique described in the previous section, and the best estimates of the glow peak shapes (determined from the non-linear least squares method, §2.1), individual glow peaks can be successively stripped so that complex glow curves can be separated into their component peaks.

2.4 Dose response of individual glow peaks

To study the supralinearity and saturation characteristics of individual glow peaks, the techniques described above have been applied to resolve the separate glow peaks (in the read-out temperature range of 100-374°C) for ^{7}LiF TLDs irradiated with gamma-ray doses in the range 10 mGy - 20 kGy. Figure 1 shows the relative peak intensity (for peaks 3.5 - 7) as a function of dose, normalised to 1 for doses < 0.1 Gy. Regions of zero slope correspond to regions of linear dose-response, regions of positive slope supralinearity and negative slope saturation. The results obtained to date are consistent with the track interaction model[4,5].

2.5 Discussion

A report describing the glow peak separation methods and their application to study the dose response of individual peaks is in preparation[6]. The techniques are being further applied to TLDs read out using UV photo-transfer (both after normal read out up to 300°C and 375°C) and, in the near future, they will be applied to study other factors affecting the glow peaks, eg cooling rate during annealing.

3. Theoretical studies of TL kinetics

The term 'TL kinetics' refers generally to the rate equations governing the filling of electron and hole traps during irradiation and the redistribution of these trapped charges during read out of the TL phosphor. A study of this topic is important because it allows a quantitative study of the following processes to be made:

(i) The concentration of trapped charges resulting from a given irradiation and its dependence upon:
  (a) the total dose;
  (b) the dose rate;
  (c) the properties (recombination coefficient, retrapping coefficient etc) of the defect centres at which the charges are localised;
  (d) the ambient temperature during irradiation.

(ii) The loss of trapped charge occurring during storage at various times at various temperatures.

(iii) The form of the glow curve produced from a given set of defect levels and trapped charges remaining at the end of irradiation and storage.

Traditionally, the above-mentioned processes have been described in terms of various approaches referred to as 1st, 2nd or general-order kinetics which involve a number of simplifying assumptions but which allow analytical solutions of the rate equations to be extracted.

The main burden of the research we have carried out over the period of this project has been to raise these simplifying restrictions and to examine all of the above-mentioned processes in more detail and solving the appropriate system of rate equations using numerical methods. Some of the main features of this work are described below.

3.1 Realistic models for TL

3.1.1 Trap filling

Trap-filling has been investigated using realistic arrays of defect centres. Detrapping and recombination during irradiation have been considered as have trap-creation and band-to-band recombinations. We have investigated:

(a) trap-filling at elevated temperatures;
(b) supralinear growth of trapped charge density including the effects of trap-creation via irradiation.

The model developed shows that the trap correction term can lead to non-linear growth in the trapping charge densities and that thermal detrapping and band to band recombination can introduce a dose-rate dependence into trap filling[7].

3.1.2 Glow curves

Realistic models of TL phosphors have been set up by specifying a set of defect centres and assigning values to trap depths, frequency factors, recombination and retrapping coefficients. It was assumed throughout that both electrons and holes could be released and redistributed during heating. The resulting TL glow curves have then been analysed using the standard glow-curve analysis techniques in order to extract the relevant trap parameters and to compare them with the values used originally as input. This work[8,9] has highlighted a number of shortcomings in the methods of glow-curve analysis. In particular, it has been shown that, given certain ranges of input-parameter values, the correct trap depths and frequency factors may not be obtained by analysis of the resultant glow curve. This work has been extended to the case of multi-peak glow curves[10].

3.1.3 Combined model for trap filling and glow curves

Recently the computer models used for calculating the kinetics of trap filling and read out have been combined into one unified model which allows the evolution of charge densities during irradiation, storage and read out to be calculated.

3.2 Non-standard TL models

Our most recent work has been concerned with the kinetics of non-standard TL models. Some phosphors exhibit TL without accompanying thermally stimulated conductivity. Presumably charge transfers from traps to recombination centres without passing through the conduction or valence bands. Modelling of the kinetics of this process shows that such a model always leads to a first-order type of glow curve[11].

Where competition exists between detrapping via the conduction band and a localised level (fig 2) the former process will dominate at fast heating rates whereas the latter is more important at low heating rates and during fading at ambient temperatures. This property has been invoked to account for anomalous fading of TL in archaeological samples[12].

4. The effect of high doses and annealing procedure on the response of Vinten Sintered LiF chips

Sintered LiF TLDs (manufactured by Vinten) are repeatedly used to monitor gamma-ray doses (~2 Gy) given to leukaemia patients undergoing radiotherapy. Some studies were undertaken to examine the effect of repeated high doses on the response of the TLDs. For TLDs repeatedly irradiated to 2 Gy and annealed at 300°C the sensitivity to a dose of 20 mGy and 2 Gy increased by 8% and 6% respectively after each increment to 2 Gy (fig 3). The zero dose reading also increased, from an initial value of 0.04 mGy, by ~0.3 mGy for each pre-exposure to 2 Gy. The dose-response relationship up to doses of 10 Gy showed that the response became supralinear above ~4 Gy.

The larger increase in sensitisation seen for the smaller test dose is not consistent with the simple competing trap model of sensitisation in which the increase would be larger for a larger test dose. Further work is in hand to investigate this effect, in particular to examine whether the sensitisation is dependent on the fractionation of the pre-exposure. None of these effects were observed with a high temperature anneal of 400°C.

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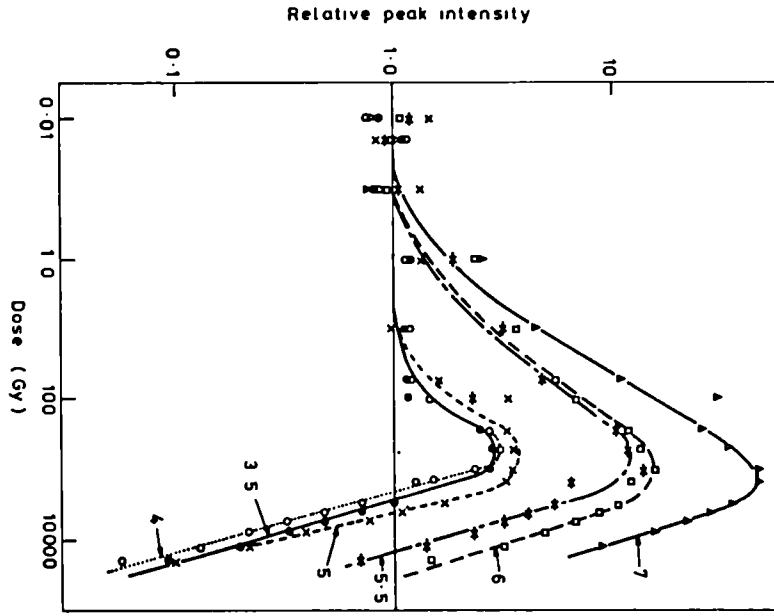

Figure 1 Relative peak intensity normalised to 1 for doses (< 0.1 Gy) against dose for peaks 3.5 to 7.

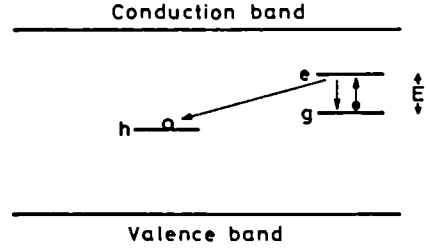

Figure 2 Localised transition model: Luminescence is produced when an electron is thermally raised from the ground state (g) to the excited state (e) from which a direct transition to the hole state (h) occurs.

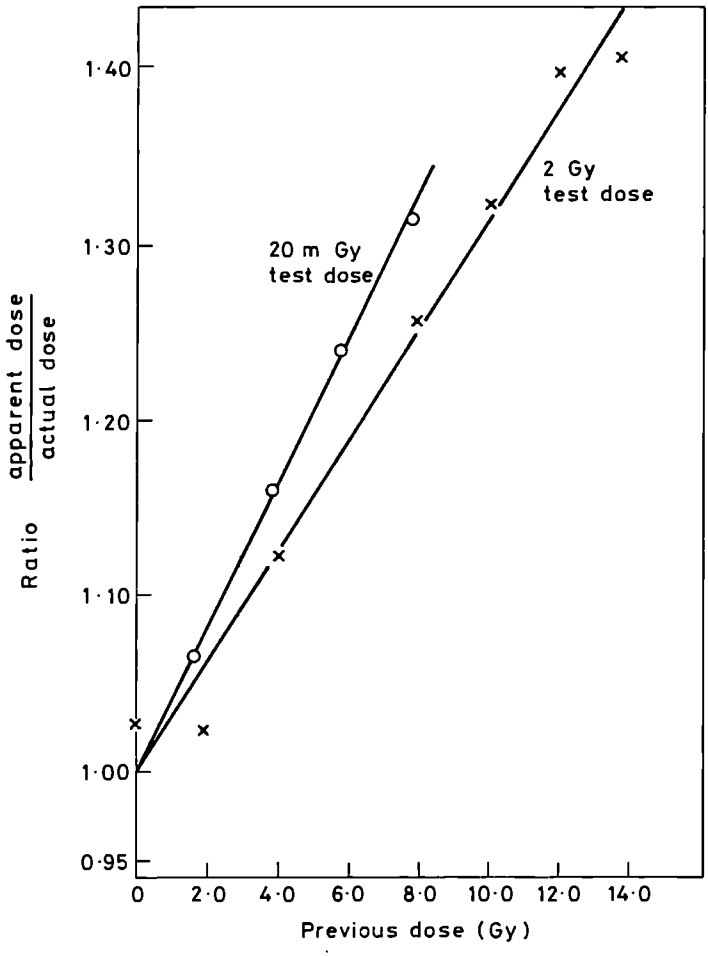

Figure 3 Effect of repeated irradiations of 2 Gy doses on the response of Vinten Scintered LiF chips annealed at 300°C.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr S W S McKeaver, Department of Physics, Oklahoma State University, Stillwater, Oaklahoma 74078, USA.

Dr R Chan, School of Physics & Astronomy, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Israel.

Dr V K Mather, Dr Joanne F. Rhodes, Dr M D Brown, Naval Surface Weapons Center, White Oak, silver Spring, Maryland 20910, USA.

V. Publications:

J A Douglas, C A Perks and A D Cotterill. The variation with LET of the response of TLD-700 and TLD-600 at temperatures up to 200°C, 8th International Conference on Solid State Dosimetry, Oxford, August 1986, Radiation Protection Dosimetry 17 (1-4), pp 479-482 (1986).

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Title of the project no. 2

Neutron Spectrometry

Head(s) of project: H J Delafield

Scientific staff: R Birch
K G Harrison
J A B Gibson

I. Objectives of the project (July 1985 - July 1988):

(a) To develop and calibrate a high sensitivity transportable neutron spectrometry system based on large H_2-filled counters with compact electronics providing simultaneous data-acquisition. The aim is to have a spectrometer, suitable for field measurements, covering the energy-range from about 50 keV to >2 MeV at dose-equivalent rates 5-50 $\mu Sv\ h^{-1}$.

(b) To develop a ^4He alpha-recoil counter to extend the neutron energy range upwards to about 14 MeV.

(c) To develop a comprehensive data analysis programme to produce the best overall neutron spectrum based on measurements with hydrogen counters, a ^4He counter and incorporating multisphere data.

II. Objectives for the reporting period (Jan - July 1988):

(i) To publish two comprehensive Harwell reports on the research and development of both the high sensitivity H_2 counter system and the ^4He alpha-recoil counter (Delafield and Birch, 1988a; Birch, 1988).

(ii) To analyse and report upon the comparison of cylindrical and small spherical H_2 counter measurements of the leakage spectrum from ASPIS shield facility at the NESTOR reactor (Delafield and Birch, 1988b).

III. Progress achieved:

1. Introduction

The measurement of the dose equivalent to personnel due to neutrons is becoming more important with the fabrication of plutonium fuels and the increased processing of high burn-up fuels containing plutonium and curium isotopes. The radiological hazard (dose equivalent) of a neutron field is a strong function of neutron energy, but the neutron spectra around both existing nuclear plants and at power stations are not well known because of the limitations of the present techniques. Hence spectral measurements are required in the working environment because the responses of both

personnel neutron dosemeters and survey instruments are not energy independent.

For such measurements, a neutron spectrometry system is required to have good energy resolution, and to be based on detectors which have both an essentially isotropic response and adequate sensitivity. Earlier work at Harwell, to establish a spectrometry system based on a set of small spherical proportional counters (type SP2, diameter 40 mm) filled with hydrogen, showed the potential of the hydrogen counter system for high resolution neutron spectrometry in radiological protection (Birch, Peaple, Delafield, Harrison and Marshall, 1985 and Birch, Delafield, Peaple and Harrison, 1985). However with an expected tightening in the regulatory dose-equivalent levels and the possibility of an increase in the quality factor for neutrons, larger-volume counters are required to increase the detection sensitivity of the system. Moreover, the spectrometry system should be made comprehensive to cover the energy ranges above the upper limit of the hydrogen counters with a ^{4}He counter and below its lower energy limit by using multispheres.

The objectives of this project were therefore, as given in Section I, to develop such a transportable neutron spectrometry system.

2. Large-volume Cylindrical Hydrogen Counters

The development and calibration of these large volume proton-recoil counters for neutron spectrometry in radiological protection has been fully described by Delafield and Birch (1988).

At the beginning of the project extensive exploratory studies were made with both large volume spherical and cylindrical counters firstly to decide upon the type of counter, and then to optimize the type and size of counter anode wire, gas fillings and the manufacturing procedure.

Cylindrical counters offered significant advantages in both construction and operating conditions enabling standard amplifiers to be used. Moreover their anisotropic response would on theoretical considerations only be expected to produce a small additional uncertainty for practical field measurements, as was subsequently verified experimentally.

Hydrogen-filled proportional counters make extremely stringent demands upon counter manufacture; in particular on the uniformity of the anode wire and purity of gas filling. Extensive studies showed that the best resolution counters were obtained using a platinum wire (40 μm diameter) and that to achieve counters with a higher upper energy-limit, xenon or argon gas could be added to increase the gas stopping power.

A set of four cylindrical counters (50 mm diameter by 300 mm long) are used to cover the neutron energy-range from about 50 keV to 2 MeV. The nominal counter filling pressures are 0.8 atm H_2, 2.7 atm H_2, 4.0 atm H_2 + 0.5 atm Xe and 2.0 atm H_2 + 3.0 atm Ar (1 atm = 101.325 kPa at 0°C). In addition, each counter contained a trace of ^{3}He gas (~0.05 kPa) to provide traceability of the energy calibration to future spectrometry measurements of unknown fields. On irradiation by thermal neutrons, an energy of ~764 keV is deposited by the ^{3}He (n,p)^{3}H reaction. Moreover since the resolution of the ^{3}He peak is very similar to the intrinsic resolution of the counter to protons, it provided a critical test of counter performance. Counter resolutions in the range 3 to 5% were achieved.

The set of cylindrical counters was calibrated using monoenergetic neutron beams at the National Physical Laboratory (NPL) to give measured response functions, energy and fluence calibrations. Monoenergetic neutrons were produced by the Van de Graaff accelerator employing the $^{7}Li(p,n)^{7}Be$ and $^{3}H(p,n)^{3}He$ reactions. The effects of neutron scatter were firstly minimised by making the measurements in a large laboratory providing low-scatter conditions and secondly corrected for by making shadow-cone measurements. The response functions were measured for side-on and end-on counter irradiations.

A Monte Carlo program was also used to calculate the response functions for comparison with the measured ones, and as a means of interpolating between the measured values and incorporating them into the unfolding codes. Good agreement was obtained between the measured and calculated response functions for both within and above the working energy-range of a counter.

A comparison was also made of the neutron fluences estimated from the measured response functions of the counter and those derived from the NPL long counter. For the side-on irradiations, the average value of the ratio of the fluences estimated for the cylindrical counters to those determined by the long counter is 1.07 ± 0.05 (SD).

3. Alpha-recoil Counter

A cylindrical alpha-recoil proportional counter has been developed to measure neutron energy spectra between 2 MeV and 15 MeV. The counter, which has an active length of 300 mm and a radius of 25 mm, is filled with 600 kPa ^{4}He plus 400 kPa Ar (Birch, 1988). The counter was made with a lead lining (thickness 0.5 mm) to reduce the effect of wall-produced protons from (n,p) reactions in the steel counter wall from neutrons of greater than 10 MeV energy. The counter was filled with a variety of test fillings to determine the optimum gas composition. To measure neutrons having energies of up to 15 MeV it is necessary to add a noble gas to increase the stopping power. Kr, Xe and Ar gases were tested. Fillings containing Kr gas were found to have a large background count due to ^{85}Kr, present in the atmosphere following fuel reprocessing. Also Kr and Xe were found to be more susceptible to gas impurities, resulting in incomplete charge collection. Ar gas did not suffer from either of these effects, and the final filling was 400 kPa of Ar plus 600 kPa of ^{4}He, sufficient to measure neutrons having energies up to 15 MeV.

The counter was calibrated with monoenergetic neutrons in the low-scatter facility of the National Physical Laboratory. The measurements provided response functions at 2.5, 3.75, 5.0 and 14.64 MeV, an energy calibration, and a fluence calibration. Subsequent measurements can be related back to the NPL calibrations by means of a ^{3}He reference peak. A reference edge is also present at 5.3 MeV due to radioactive contaminants in the lead and solder components of the counter.

Since it is not possible to generate monoenergetic neutron beams between ~6MeV and 12 MeV, the Monte Carlo code HELIUM85 was written and used to interpolate between the measured response functions. This code was written to calculate the response of a cylindrical counter to monoenergetic neutrons. The program includes wall and end effects, elastic scattering with ^{4}He and argon nuclei, and inelastic interactions

with ^{40}Ar. The program showed that the end effect, due to the fall-off of electric field near to the end of the counter, is small as long as the length of the counter is large compared to its radius.

Good agreement was obtained between the measured and calculated response functions and so HELIUM85 was used to generate the response function matrix necessary for unfolding the neutron spectrum from the measured pulse-height distribution. A number of unfolding methods were investigated. To test the various techniques, several responses to monoenergetic neutron beams of different energies were added together. A matrix inversion technique gave fairly good estimates of the neutron fluence for each energy but had the disadvantage of giving broad resolution peaks. An iterative technique was tested and gave better resolution of the peaks since the effect of counter resolution can be included in the response functions. Differentiating both the response function matrix and the measured pulse-height distribution gave even better resolution of the peaks, and better estimates of the neutron fluence. This method was used to unfold all subsequent measurements.

Unfolding the measured response functions obtained at the NPL, for which measured fluences were also available, showed that the fluences measured using the alpha-recoil counter were within $\pm 8\%$ of the quoted NPL values for side incidence.

4. Spectrometry Measurements

A number of neutron spectrum measurements were then undertaken with both the cylindrical hydrogen-filled counters and the alpha-recoil counter to ensure that the unfolding programs, which had been developed to derive the spectrum from the measured pulse-height distribution, were operating satisfactorily.

Delafield and Birch (1988) made measurements with the cylindrical hydrogen-filled counters at different angles of incidence to the neutron source for the leakage spectrum of fission neutrons through the NESDIP3 shield at the NESTOR reactor (AEE Winfrith). In addition, a direct comparison has been made of spectrum measurements (NESDIP3 shield and an ^{241}Am-Li (α,n) source) with those obtained using the smaller spherical counters (type SP2).

The angular measurements showed that an essentially isotropic response can be achieved in practical field measurements by using the cylindrical counters in an orientation such that neutrons will be incident predominately upon the side-walls of the counter, at approximately normal (90^{o}) incidence to the counter axis. The neutron spectrum from an ^{241}Am-Li source measured with the cylindrical counters under low scatter conditions, was in good agreement with a previous measurement made with the spherical counters (Fig 1). For the NESDIP3 shield, the cylindrical and spherical counter measurements of the spectrum were in reasonable agreement and the derived dose-equivalent from the cylindrical counters was about 0.93 of that given by the spherical counters and an organic scintillator.

A test facility for the hydrogen counters comprising an ^{241}Am-Be (α,n) source, a moderator and absorption filters to produce resonance spectra (e.g. ^{19}F, ^{28}Si and ^{16}O) has also been successfully demonstrated.

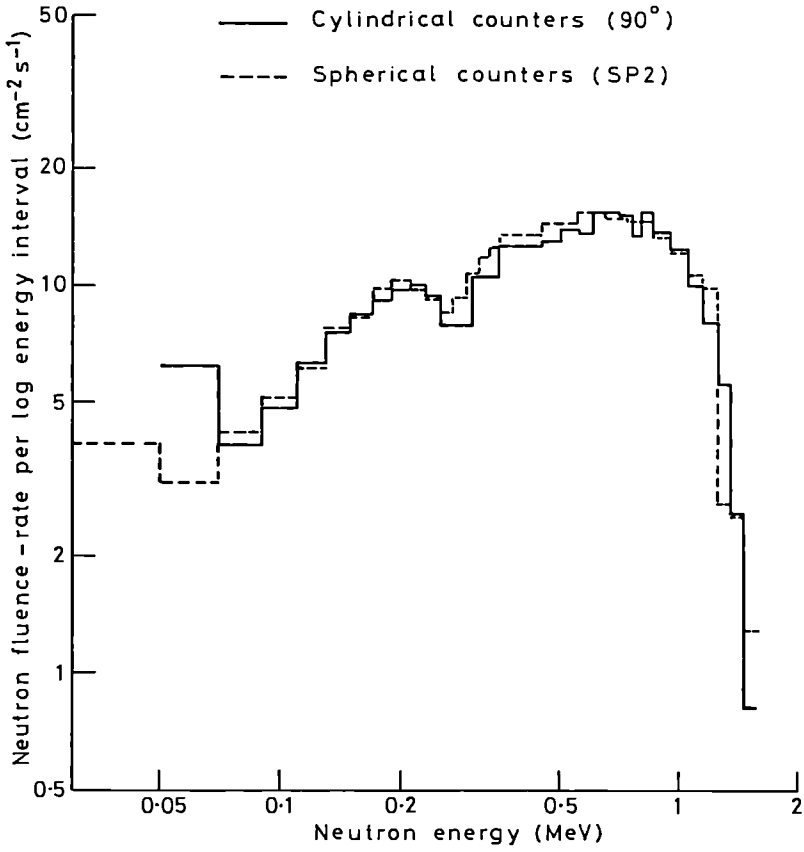

FIG.1 COMPARISON OF NEUTRON SPECTRUM OF ^{241}Am-Li SOURCE MEASURED WITH CYLINDRICAL AND SPHERICAL PROTON - RECOIL COUNTERS.

Likewise, measurements made by Birch (1988) with the alpha-recoil counter of neutron spectrum from an Am-Be source (Fig 2), and of the leakage spectrum from the NESDIP3 shield of the NESTOR reactor showed very good agreement with other measurements and calculations.

Finally the ability to measure a neutron spectrum over the complete energy range of interest down to thermal energies was demonstrated by measurements made of the neutron spectrum inside the containment walls of a PWR (Birch, Delafield and Perks 1988). In this case, on account of the higher dose-equivalent rate, the measurements were made with the lower sensitivity SP2 hydrogen counters, the alpha-recoil counter and a set of multispheres. The solution for the multispheres was constrained by the spectrum measured above about 50 keV with the hydrogen counters.

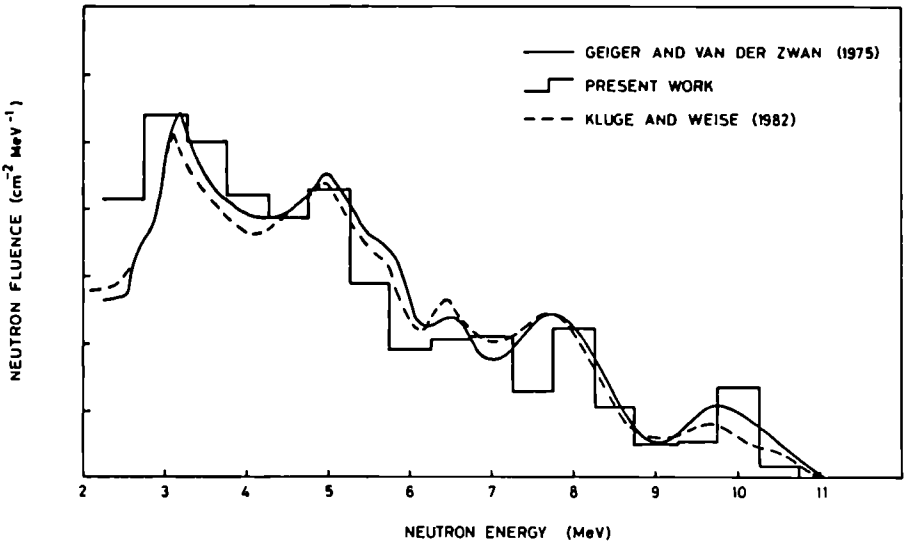

FIG.2. NEUTRON SPECTRUM FROM AN Am-Be SOURCE MEASURED USING THE ^{4}He RECOIL COUNTER.

5. Conclusions

A transportable neutron spectrometry system of high sensitivity, based on large-volume cylindrical proton-recoil proportional counters, has been developed for the high-resolution measurement of neutron spectra from about 50 keV to 2 MeV in the working environment at dose-equivalent rates in the range 5-50 μSv h^{-1}. The system uses modern compact electronics to provide simultaneous data acquisition with a single multichannel analyser. An alpha-recoil counter, which uses identical electronics to that used for the proton-recoil counters, has been developed to measure the neutron spectrum in the energy range from 2 to 15 MeV.

Both the proton- and alpha-recoil counters have been calibrated at the NPL using monoenergetic neutrons to provide measured response functions, energy and fluence calibrations. In addition, a Monte Carlo code was developed to calculate the response functions for both types of counters and to interpolate between measured response functions. In both cases good agreement was obtained between the measured and calculated response functions.

Finally measurements were made of the leakage spectrum from the NESDIP3 shield of the NESTOR reactor and of the radionuclide sources Am-Li (H_2 counters only) and Am-Be (alpha-recoil counter only), which demonstrated that the unfolding programs operate very satisfactorily. In addition, measurements made with hydrogen counters at different angles of incidence using the NESTOR facility showed that they had an essentially isotropic response.

The complete spectrometry system comprises the hydrogen counters, the alpha-recoil counter to extend the energy-range up to 15 MeV and a set of multispheres to measure the dose-equivalent below 50 keV.

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Kluge, H. and Weise, K. (1982). The neutron energy spectrum of a ^{241}Am-Be(α,n) source and resulting mean fluence to dose equivalent conversion factors. Radiation Protection Dosimetry. 2(2), p 85.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. ^{4}He counter developed in co-operation with Dr M C Scott, Department of Physics, University of Birmingham

2. Collaborative measurements with Dr C Wernli and Dr C Barth (EIR, Wurenlingen, Switzerland) of neutron spectra inside containment building of PWR

3. Collaboration through Eurados-Cendos (partial funding of Gosgen PWR measurements)

4. Contacts were made during the project with both Dr J Chartier (CEA-FAR) and Dr K Knauf (PTB, FRG) who are undertaking similar work

V Publications:

1. Scientific Journals

Birch R, Peaple L H J, Delafield H J, Harrison K G and Marshall M (1985) Development of hydrogen proportional counter spectrometry for radiological protection. Proceedings of 5th Symp. on Neutron Dosimetry, Neuherberg, W Germany. EUR 9762 EN Vol 1, p 369.

Birch R, Delafield H J, Peaple L H J and Harrison K G (1985). The neutron leakage spectra through the steel top plates of two heavy-water-moderated research reactors, Radiation Protection Dosimetry. 12, p 285

Perks C A, Harrison K G, Birch R and Delafield H J. (1986). The characteristics of a high intensity 24 keV iron-filtered neutron beam. Radiation Protection Dosimetry. 15.,p 31.

Birch, R., Delafield, H.J. and Perks, C.A. (1988) Measurement of the neutron spectrum inside the containment building of a PWR. Proceedings of the 6th Symp. on Neutron Dosimetry, Neuherberg, W Germany Oct 12-16, 1987 Radiation Protection Dosimetry. 23, Nos. 1-4., p 281.

2. Reports

Delafield, H J and Birch, R (1988a). Development and calibration of large volume proton-recoil counters for neutron spectrometry in radiological protection. AERE-Report R13010.

Birch, R (1988). An alpha-recoil proportional counter to measure neutron energy spectra between 2 MeV and 15 MeV. AERE-Report R13002.

Delafield, H J and Birch, R (1988b). Neutron spectrometry measurements with large volume cylindrical proton-recoil counters developed for use in radiological protection. AERE Report R13103 (In press).

Title of the project no. 3

Development of a CR-39 Personnel Neutron Dosemeter

Head(s) of project:

K G Harrison

Scientific staff:

R J Goodenough

I. Objectives of the project:

To explore improvements which can be made in neutron dosemeters (lower energy and dose threshold, better reproduceability and flatter dose-equivalent response), using CR-39 plastic processed by electrochemical etching, and to determine the practical limitations for routine personnel dosimetry. Plastic samples manufactured under new and improved conditions will be tested for neutron response (energy and angle), background, ageing, fading and environmental effects.

II. Objectives for the reporting period: (1985-1987)

(i) To determine the effects of time and environment on the background and neutron sensitivity of electrochemically-etched CR-39 from various sources, and to seek causes of changes and ways to minimize those which are undesirable.

(ii) To simplify handling and processing procedures for an eventual full operational service.

(iii) To measure the energy and angular response of the elements of the dosemeter.

(iv) To hold the European Workshop on the "Development of Personnel Neutron Dosemeters Based on (Proton-Sensitive) Track Etch Detectors".

(v) To determine the optimum solution to the problem of angular response.

III. Progress achieved:

Progress during 1985

Work during 1985 concentrated on determining environmental and temporal influences on background and neutron sensitivity, and comparative studies between commercial CR-39 (Pershore Mouldings Ltd) and newly standardised CR-39 from Bristol University. Much of this work was reported at the International Solid State Nuclear Track Detector Conference (Rome), in September 1985[1]. Other work included improving and scaling-up processing equipment and simplifying procedures for an eventual operational service; all this work was reported in detail elsewhere[4,5].

The first environmental studies involved the storage of irradiated and unexposed CR-39 samples, from Pershore Mouldings Ltd and Bristol University, at room temperature and -20°C in air, and at room temperature in a dry enriched nitrogen atmosphere, for 6-8 months.

A further study of the rate of increase of background on CR-39 samples when stored beneath an airspace under several thicknesses of polythene (0-150 μm) was also undertaken, using samples taken from two sheets of plastic (of different ages) from Bristol University. The results of this study showed clearly that 100 μm of close-fitting polyethylene is necessary to give a very high level of protection from radon.

Studies involving the storage of unexposed and irradiated CR-39 samples in air at two temperatures (15°C and 40°C) and four humidities (0, 56, 76, 100%) for periods of 3-6 months and similar studies on the effects of darkness and daylight were also started in 1985 (and completed in 1986).

A study was undertaken to determine the range of linearity and the dispersion of measurements at different doses of ^{252}Cf fission neutrons, between 0.5 mSv and 24 mSv, with some samples processed in one batch and some in several batches.

Finally, a new processing regime was developed and tested, in which both pre-etching and electrochemical etching are undertaken at the same temperature (35°C), but the same thickness of bulk material is removed in each step as in the original processing regime.

Results obtained during 1985

Experiments involving storage of irradiated and unexposed CR-39 in air and nitrogen showed some loss of sensitivity for those samples previously stored in air at room temperature for ~ 6 months, although loss of existing latent tracks was negligible for all three storage conditions. An increase in background with time was observed, which seemed to be similar for all three storage conditions. The Bristol plastic has generally shown slightly less changes with time.

The loss of sensitivity with time is quite small, and although storage in dry nitrogen inhibits the effect, we believe that tracks

need to be oxygenated, during or possibly after formation, or they are not etchable (see (4)), so storage in nitrogen must be employed with care.

Results obtained from storage of plastic under several thicknesses of polyethylene indicated that 50 μm thick coverings were not completely effective in shielding environmental alpha radiation, but 100 μm was very effective.

The linearity study showed the dose-response to be linear up to ~ 10 mSv, but at 24 mSv it was ~ 15% low. Dispersions were only slightly greater than Poissonian (up to a factor of ~ 1.5), with no differences observed for batches processed together and separately.

The new processing regime (pre-etch 6 h: 6.25N KOH, 35°C; ECE 6 h: 6.25N KOH, 35°C, 2 kHz, 21 kV(rms) cm^{-1}) gave the same results for AmLi, ^{252}Cf and AmBe neutrons and background (within experimental uncertainties) as the older process.

Progress during 1986

It was found in 1985 that changes in the manufacturing process of the Pershore plastic had led to low-background high-sensitivity material, which does not show significant increase in background with time. However, the plastic has continued to show a slow (but tolerable) loss of sensitivity to neutrons with time, when stored in air. Furthermore, the poor angular response of the single planar element dosemeter was identified as a major shortcoming, and work during this year has concentrated on these two problems.

Results obtained during 1986

Irradiations of mock-up dosemeters with two or three mutually-orthogonal detectors demonstrated the superiority of these arrangements, but it would be operationally simpler and cheaper to use an angular-averaged sensitivity for a single element. A full irradiation programme of neutron energy and angular responses on phantom was undertaken in July to complete the experimental requirements to allow an optimum solution to this problem to be determined. Samples were processed using the 6 h pre-etch/ 6 h ECE regime, and also using a 3 h pre-etch/9 h ECE regime which is closer to that proposed by Tommasino to improve the energy response and sensitivity. It was found that, while there was some improvement in the energy response, there was an accompanying important improvement in the angular response.

Experiments on the combined effect of temperature and humidity, and also the effect of daylight and UV, on the sensitivity and change of background with time, as well as the fading of tracks, were completed and these showed no strong effects. Measurements of the effect of temperature during registration on the sensitivity of the plastic were also completed and showed only a small effect. This work was reported in a paper at the Solid State Dosimetry Conference (Oxford, August 1986)[2].

We participated in European/US/Canadian joint irradiations of CR-39 dosemeters in November, organised by Eurados/Cendos Committee 5. The

dosemeters were sent by post by 14 participating dosimetry laboratories to four irradiation laboratories, to be exposed to monoenergetic neutrons (thermal - 14.8 MeV) and ^{252}Cf fission neutrons. The results of these joint irradiations were published in a KfK report[7].

Progress during 1987

Work during the first part of the year concentrated on completion of the processing and analysis of CR-39 samples irradiated in the Joint European/US/Canadian Irradiations in November 1986, and a comparison between these results and those obtained in a comprehensive set of calibration irradiations at the National Physical Laboratory in July 1986. In addition, long term high-humidity room temperature ageing/fading studies were completed early in the year. All this work was to have been written up for a paper in the Neutron Dosimetry Symposium at Neuherberg, but this was not completed in time. However, it will be written up as part of the case for formal approval of an operational neutron dosimetry service at Harwell, which commenced pre-operational trials on 1 January 1988 (but has continued to give unsatisfactory results because of the poor angular response of a single planar element).

Effort was expended on the organisation of the Harwell/CEC/Eurados-Cendos Workshop on the Development of Personal Neutron Dosemeters Based on (Proton-sensitive) Etched-Track Detectors, which took place at Harwell over the three days of 12-14 May 1987. Forty-seven scientists from fourteen countries participated in the Meeting, at which twenty-four papers were presented and discussed. These papers were published in a special issue of the Journal Radiation Protection Dosimetry[3].

The impression gained at the Meeting was that simple neutron dosemeters based on CR-39 are close to being acceptable for routine use, with some doubt about their angular response. Although variable backgrounds and sensitivity are under reasonable control in present supplies of plastic, the reasons for these variations are not well-enough understood to allow complete confidence in the quality of future material.

The Workshop undoubtedly achieved its aim of clarifying the state of the art in this subject and its conclusions are endorsed by the work undertaken in this project.

This project terminated in 1987.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Close discussions on progress maintained with members of the UK Track Etch Group (Bartlett, NRPB, Chilton; Harvey, CEGB, Berkeley, UK; Henshaw, Univ. Bristol) and with members of the EURADOS-CENDOS Track Etch Group: (Bartlett, NRPB, Chilton, UK; Decossas, Univ. Limoges, France; Harvey, CEGB, Berkeley, UK; Lembo, ENEA, Bologna, Italy, Medioni, CEA, Fontenay, France; Piesch, KfK, Karlsruhe, FRG; Schraube, GSF, Neuherberg, FRG and Tommasino, ENEA, Rome, Italy). Hollnagel, PTB, Braunschweig, FRG is also collaborating on this work through the EURADOS-CENDOS Group.

V. Publications:

(Publications in scientific journals)

1. "Some studies of the neutron response, ageing and fading properties of two different types of CR-39 plastic processed by electrochemical etching", K G Harrison, R M Haigh and R J Goodenough, Proc. 13th ICSSNTD, Nucl. Tracks, 12, 653-656 (1986).

2. K G Harrison and R J Goodenough, "Process Towards an Operational Personnel Neutron Dosemeter Based on Electrochemical Etching of CR-39", Proceedings 8th International Conference on Solid State Dosimetry, Oxford (1986) Rad. Prot. Dos. 17, 143-147 (1986).

3. Proceedings of a Workshop on "Etched Track Neutron Dosimetry" (Harwell (UK) May 1987), published as Vol 20 Nos 1 and 2, Radiation Protection Dosimetry (1987) (Editors: D T Bartlett, J Booz and K G Harrison).

(Reports)

4. "Operational trials of a CR-39 neutron dosimetry system: progress to January 1985", K G Harrison, R M Haigh, R Turner and R J Goodenough, AERE Report R-11808 (21985).
5. "Neutron irradiations of proton-sensitive track detectors: results of a joint irradiation organised by CENDOS", K G Harrison (editor), AERE Report R-11926 (1985).

6. K G Harrison and R J Goodenough, "Operational trials of a CR-39 neutron dosimetry system: progress to January 1986", Harwell Report AERE R-12278, (1986).

7. R J Goodenough and K G Harrison, "Summary of Results obtained at Harwell using Electrochemical Etching of Commercial Grade CR-39" in EURADOS-CENDOS Report 1987-01 published by KfK as KfK 4305 (Editor: E Piesch), September 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Medical Research Council
20 Park Crescent
GB London W1N 4AL

Contract no.: BI6-A-009-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. D.T. Goodhead
MRC Radiobiological Unit
Cell and Molecular Biology Div.
Harwell, Didcot
GB Oxon OX11 ORD

Telephone number: 235-83.43.93

Title of the research contract:

Biophysical studies of relations between radiation dose, quality and biological effect.

List of projects:

1. Analysis of physical properties of diverse radiations in relation to their observed biological effectiveness.
2. Development of radiation sources and dosimetric techniques for radiobiological studies at low and high dose-rate.

Title of the project no.: 1

Analysis of physical properties of diverse radiations in relation to their observed biological effectiveness.

Head(s) of project: Dr. D.T. Goodhead

Scientific staff: Dr. D.T. Goodhead
Dr. H. Nikjoo
Dr. J. Thacker
Dr. D. Stevens

I. Objectives of the project: This project is to generate and analyse relevant physical and biological data and to deduce the implications for radiation protection. The specific aims are:

a) Calculation and comparison of local energy deposition by radiations of different qualities in target structures of varying size and shape including simple models of biological macromolecules such as the DNA duplex and higher order DNA structure.

b) Undertaking of in vitro biological experiments to supplement and extend the data already available on the biological effectiveness of diverse radiations in inducing relevant cellular effects.

c) Comparison of physical properties of radiation energy deposition with observed biological effectiveness, so as to identify regions of volume and energy which do or do not, correlate with biological effectiveness.

II. Objectives for the reporting period:

1) Continue scoring and tabulation of absolute frequency distributions of energy deposition in small cylindrical volumes by mono-energetic electrons of 0.1 to 100 keV and combine these for representative hard X-ray spectra; improve statistics by application of a Cray XMP48 supercomputer. Consider relativistic extension of the Monte-Carlo electron code to >100 keV.

2) Characterization of sensitivity of repair-deficient mutant lines of V79 hamster cells to α-particles and low-LET radiations, investigate effects of low dose-rate α-particles on hamster cells (killing, aberrations and possibly mutation) and on 10T½ cells (transformation), and consider direct comparison of effectiveness of cyclotron-produced α-particles and protons.

III. Progress achieved:

1. Track structure implications for very small targets

We have used the track structure codes MOCA-8 (electrons) and MOCA-14 (protons and α-particles) of Paretzke and Wilson to extend our data-base, and make comparisons, of the absolute frequency distributions of energy deposition in very small cylindrical targets by different radiations. Full scoring is now complete for selected mono-energetic electrons representing the energy range from 0.1 to 100 keV and cylindrical target sizes from 1 to 100 nm diameter (d) and lengths d/2 to 4d. The scoring code was successfully installed and operated on the Rutherford Appleton Laboratory CRAY XMP/48 computer in addition to our own Norsk Data microcomputer so that good scoring statistics could be obtained for the higher energy electrons whose interactions are very widely distributed in space. Multiple-stage scoring was again used to increase further the efficiency of scoring but to retain accurate absolute normalization of the distributions in terms of total incident, or absorbed, energy.

These frequency distributions of energy deposition by electrons can be compared directly with those previously computed by us, using similar methods, for ultrasoft X-rays (0.28-4.5 keV), protons (0.3-4.0 MeV) and α-particles (1.2-20.0 MeV) for the variety of diameters (1-100nm) and aspect ratios (0.5-8) of the targets. We have to date made fairly detailed comparisons for a few particular selected cylinders, corresponding approximately to a short segment of DNA (d=l=2 nm), a nucleosome (d=10 nm, l=5 nm) and a short segment of chromatin fibre (d=l=25 nm) and for particular radiations, namely ultrasoft X-rays (C_K, Al_K and Ti_K of 0.28, 1.5 and 4.5 keV, respectively), 0.5 MeV protons (to represent intermediate-LET proton-recoils from fission neutrons), 3 MeV α-particles (to represent high-LET tracks from natural α-emitters) and 100 keV electrons (to represent low-LET radiations). Interesting features revealed by the comparisons include: the relatively large numbers of large direct energy depositions which occur in all such targets in a single mammalian cell compared to the biologically observable phenotypic damage; the uniquely large deposition which can be produced by high-LET radiations but which are virtually unachievable by low-LET radiations; the almost total lack of multiple-track effects in these small targets; the fairly close correspondence between the absolute numbers of $\geq$ 100 eV depositions in DNA segments and the measured numbers of double-strand breaks, and the much smaller numbers of lethal events; and the expectation that RBE's of ultrasoft X-rays will be considerably reduced in more radio-sensitive cells if their sensitivity is due to them responding to smaller local concentrations of energy.

Calculations have been carried out on the probabilities of DNA single- and double-strand breakage by the different radiations, in collaboration with D.E.Charlton who has developed a model for this process by direct action. This considers the internal spatial structure of individual energy deposition events in DNA and assumes, based on fits to observed breakage of single-stranded DNA by iodine-125 in experiments by Martin and Haseltine, that deposition of 17.5 eV in a single sugar-phospate moiety causes a strand break. Absolute calculated yields of breaks were compared with experimentally measured yields in the literature. Good agreement was obtained for yields and radiation quality dependence for single-strand breaks, but for double-strand breaks the calculated yields were greater than those measured and showed less dependence on radiation quality than did some of the experimental data.

2. Biological consequences of radiation quality and dose-rate

Further experimental data have been obtained on the effectiveness of radiations of different qualities, and of well defined track structures, in inducing relevant cellular effects. Dose-rate effects with slow α-particles have also been investigated.

The sensitivity of three V79 hamster cell mutant lines (irs-1, irs-2 and irs-3) to low energy (3.2 MeV) α-particles has been ascertained. These different mutants were previously isolated by Jones and Thacker who showed that they were two to three-fold more sensitive that the parent line to low-LET radiations. We have now established that all three mutants are also more sensitive than the parent strain to these high-LET (124 keV μm^{-1}) α-particles, but that the sensitivity factor is somewhat reduced. The survival data and associated measurements, are now sufficiently accurate to allow meaningful analysis of the sensitivity in terms of particle track structure, using the track structure data generated in (1) above.

Accurate experiments are in progress to establish the degree, and direction, of any dose-rate dependence of biological effectiveness of high-LET α-particles. Inactivation of V79 cells appears to show a small reduction with reduced dose-rate, but the full statistical significance of this has yet to be evaluated. Corresponding experiments on induction of chromosome aberrations in V79 cells are well advanced but no preliminary, or final, result can be obtained until the full set of coded slides have been scored (blind). These dose-rate investigations with α-particles require cells to be grown as plateau-phase monolayer cultures in special sealed dishes which have very thin polyester bases and are individually gassed continuously as they rotate on a horizontal wheel for hours or days over the α-particle source. It was found necessary to construct the dish walls of titanium, rather than stainless steel which had unfavourable effects on cell growth under these conditions. Further experiments on dose-rate effects on $10T\frac{1}{2}$ cells have continued in collaboration with C.J.Roberts (Harwell Laboratories).

A detailed experimental plan was drawn up for direct comparison of the biological effectiveness of protons and α-particles of identical stopping-powers (in the region 20-30 keV μm^{-1}). This is to address the results recently obtained by Belli et al which apparantly show substantially greater effectiveness of protons when compared to the data in the literature for α-particles. Collaborative experiments will be undertaken in early 1989 on the Harwell Cyclotron which can produce both required particles. Implications of the results will then be considered both in terms of the subtle differences in track structure and implications for understanding effects of neutrons.

A mechanistically interesting variation in radiation track structure, and its biological implications, was provided by the 24 keV filtered epithermal neutron beam developed at the Harwell Laboratories. We have applied this to irradiation of mouse marrow (CFU's assay) and human blood lymphocytes (chromosome aberrations, micronuclei and sister-chromatid exchanges) for both of which we have comparative data for α-particles and X-rays. It was found that these neutrons had high relative biological effectiveness, despite their low energy and short recoil tracks, and that they were capable of producing SCE which is apparantly a property unique to high-LET, as opposed to low-LET, radiation.

IV. Objectives for the next reporting period:

1) Extend scoring of absolute frequency distributions of energy deposition in small cylindrical volumes to include Cu-K X-rays, additional mono-energetic electrons, X-ray spectra. Compile full scoring tabulations in report form. Consider further extension of the data base to include neutrons.
2) Continue in vitro experiments on effectiveness of low energy α-particles, including dose-rate effects and mutagenesis, low-energy neutrons, ultrasoft X-rays and direct protons/α-particle comparisons.
3) Apply data from track structure analysis to interpretation of observed biological effectiveness of the different radiations.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

M. Belli, Instituto Superiore di Sanità, Rome, Italy.
D.E. Charlton, Physics Dept. Concordia University, Montreal, Canada.
J.H. Humm, Division of Experimental Pathology & Therapeutics, MRC Radiobiology Unit, Chilton, U.K.
A.Mill, CEGB Berkeley Nuclear Laboratories, Berkeley, U.K.
H.G. Paretzke, GSF, Neuherlerg, F.R.G.
M.R. Raju, Life Sciences Division, Los Alamos National Laboratory, U.S.A.
C.J. Roberts, UKAEA Harwell Laboratories, Didcot, U.K.
W.E. Wilson, Radiological Physics, Battelle Pacific Northwest Laboratories, U.S.A.

VI. Publications:

Full Papers

Z.S.Aghamohammadi, D.T.Goodhead and J.R.K.Savage
"Induction of sister chromatid exchanges (SCE) in human lymphocytes by plutonium-238 α-particles". Int.J.Radiat.Biol. 53, 909-915 (1988)

D.T.Goodhead
"Spatial and temporal distribution of energy deposition and possible implications for radiation protection". Health Phys. 55, 231-240 (1988)

H.Nikjoo, D.T.Goodhead and D.E.Charlton
"Energy deposition in small cylindrical targets by ultrasoft X-rays". Phys.Med.Biol. (in press)

D.T.Goodhead and H.Nikjoo
"Track structure analysis of ultrasoft X-rays compared to high- and low-LET radiations". Int.J.Radiat.Biol. (in press)

J.S.Bedford and D.T.Goodhead
"Breakage of human interphase chromosomes by alpha-particles and X-rays". Int.J.Radiat.Biol. (in press)

M.E.Schillaci, S.Carpenter, M.R.Raju, R.J.Sebring, M.E.Wilder and D.T.Goodhead
"Radiobiology of ultrasoft X-rays II. Cultured C3H mouse cells (10T½)". Radiat.Res. (in press)

Z.S.Aghamohammadi, D.T.Goodhead and J.R.K.Savage
"Production of chromosome exchange aberrations, micronuclei and sister chromatid exchanges by 24 keV epithermal neutrons in human G_0 lymphocytes". Int.J.Radiat.Biol. (in press)

H.Nikjoo and D.T.Goodhead
"The RBE's achievable by high-and low-LET radiations". Proc. L.H. Gray Conference, Oxford, Sept. 1988 (in press)

D.E.Charlton, H.Nikjoo and J.L.Humm
"Calculation of initial yields of single and double strand breaks in cell nuclei from electrons, protons and alpha-particles". Submitted to Radiat.Res.

D.T.Goodhead
"Relationships of radiation track structure to biological effect: A re-interpretation of the parameters of the Katz model". Submitted to Radiat. Measurements Nucl. Tracks.

Short Communications

D.T.Goodhead, J.R.K.Savage and Z.S.Aghamohammadi
"Do high-LET radiations have infinite RBE for induction of sister chromatid exchanges (SCE) in human lymphocytes". In: Abstracts of Papers for Thirty-Sixth Annual Meeting of the Radiation Research Society, Philadelphia, June 1988. (Radiat.Res.Society, Philadelphia) p.141

Title of the project no.: 2

Development of radiation sources and dosimetric techniques for radiobiological studies at low and high dose-rate.

Head(s) of project: Dr. D.T. Goodhead

Scientific staff: Dr. D.T. Goodhead
Dr. H. Nikjoo

I. Objectives of the project:

This project is to develop experimental technologies and methodologies of special radiation sources for quantitative investigation of relevant biological effects and their interpretation. The specific aims are the investigation and optimization of conditions of production of ultrasoft, and intermediate energy, X-rays, especially by proton bombardment. This should include assessment of a variety of monoenergetic beams which can be produced, their accurate measurement and their applicability to investigate problems in radiation biology and biochemistry.

II. Objectives for the reporting period:

1. Further development of ultrasoft X-ray sources, including preliminary experiments with laser-produced plasmas, and consideration of methods for reduced dose-rates.
2. Measurements of thickness of living cells, under conditions of irradiation, by means of laser confocal microscopy and comparison with fixation and electron microscopy.

III. Progress achieved:

A number of different types of ultrasoft X-ray source are now in use for radiobiological investigations in laboratories in the U.K., U.S.A., Japan and Germany. These include cold-cathode and hot-filament electron-bombardment sources, proton-bombardment sources and synchrotrons. Published radiobiological results from the different laboratories are essentially consistent with one another. They have confirmed the original results from our laboratory showing the biological effectiveness of ultrasoft X-rays despite the very short ranges of their secondary electrons. In particular, similar results for Al-K X-rays on hamster cells have now been published by 4 laboratories, for C-K X-rays on hamster cells in 2 laboratories and C-K on yeast in 2 laboratories.

The major uncertainty in detailed mechanistic interpretation of these results, especially for the lowest energy (C-K) X-rays, arises from the rapid attenuation of the X-rays through a single attached cell. Physical absorbed dose at the incident surface of the cell can be accurately measured and specified. However, description of the radiobiologically relevant dose beyond this requires information, or assumptions, on the thickness of the cell and the intra-cellular distribution of relevant target material. The hamster cells are relatively thick (~4-7 μm) and recent collaborative investigations with Raju et al. at Los Alamos have not revealed the simple dependence with cell thickness which might have been expected for exponential attenuation through a uniform distribution of target material in the cell nucleus. Thinner cells, such as 10T½ mouse and AG1522 human cells show substantially reduced RBE's of ultrasoft X-rays when evaluated in this way. Co-incidentally, the thickness of the particular cell lines used correlates with their resistance to penetrating low-LET radiations, such as γ-rays, so it is at present not clear whether the apparant reduction in RBE with reducing cell thickness is really due to the assumptions for dose-averaging or whether it is reflecting a true mechanistic association with cell sensitivity. For example, if a particular cell type is more sensitive because it responds to smaller very local intra-track concentrations of energy deposition, then it would be expected to have a reduced RBE of ultrasoft X-rays relative to standard low-LET radiations. This expectation follows clearly from the track structure analyses described in Project 1. On the other hand, explaining the apparent reduction in RBE in terms of attenuation and distribution of target material can reconcile the data for the various cell types only by making very strange assumptions as to the distribution of target material.

In view of the above it is now of central importance to carry out accurate population measurements of the thickness profiles of attached living cells under the exact conditions of irradiation with ultrasoft X-rays. Fixation, embedding and subsequent measurements by optical or electron microscopy provide high precision but leave open the question of possible distortion by the preparation procedures. We have investigated numerous possible methods of making these measurements on living cells, but found that most are inadequate. In collaboration with N.White and S.Townsend a method has now been developed based on laser confocal microscopy. A Biorad MRC-Lasersharp confocal microscope is now being installed in our laboratory so that we can optimise the methods for our problem and obtain the essential quantitative data.

To extend the types of radiobiological investigations which can be undertaken with ultrasoft X-rays, studies are in progress to evaluate

methods for high- and low- dose-rate sources. High-intensity single pulses of ultrasoft X-rays can be produced by bombardment of targets with laser pulses to produce X-ray-emitting plasmas characteristic of the target material and the plasma temperature. In collaboration with F.O'Neill, the physical and biological properties are being determined of ultrasoft X-rays produced by bombardment of a rotating Fe target with a 0,7 J/pulse KrF laser. The mean energy of the X-rays is approximately 0-9 keV and they have been found to have similar biological effectiveness (per unit absorbed dose at the incident surface of the cells) for killing of V79 hamster cells as has been found for C-K X-rays.

IV. Objectives for the next reporting period:

1. Investigations will continue on methods of producing ultrasoft X-rays at different dose-rates.
2. Methods will be optimised for laser confocal microscope measurements of thickness profiles of living cells under exact conditions of irradiation; population measurements will be obtained and compared with conventional methods of fixation and electron microscopy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

D.Frankenberg, GSF, Frankfurt, W.Germany
F.O'Neill, Rutherford Appleton Laboratory, Chilton, U.K.
M.R.Raju, Life Sciences Division, Los Alamos National Laboratory, U.S.A.
A.F.Smith and E.Jones, St. Bartholomew's Medical College, London, U.K.
S.Townsend, AMTS Division, MRC Radiobiology Unit, Chilton, U.K.
N.White, Zoology Dept., Oxford University, Oxford, U.K.

VI. Publications:

E.A.Jones, F.A.Smith, D.T.Goodhead and J.Oriel
"Some optimum conditions for proton induced ultrasoft X-ray production".
Phys. Med. Biol. 33, 1385-1397 (1988)

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Risø National Laboratory
DK-4000 Roskilde

Contract no.: BI6-A-028-DK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Hansen
Risø National Laboratory
Accelerator Department
DK-4000 Roskilde

Telephone number: 2-37.12.12

Title of the research contract:

Investigation of alanine as an accident dosimeter and interpretation of dose-effect relationships by model description.

List of projects:

1. Investigation of alanine as an accident dosimeter and interpretation of dose-effect relationships by model description.

Title of the project no.: BI6-A-028-DK

Investigation of alanine as an accident dosimeter and interpretation of dose-effect relationships by model description.

Heads of the project:

J.W. Hansen and K. Sehested

Scientific staff:

Johnny W. Hansen and Kjeld J. Olsen

I. Objectives of the project:

The amino acid alanine is a very versatile dosimetric material with properties appropriate for an accident dosimeter. The project concerns development of the alanine dosemeter as a personal accident dosemeter and to investigate its properties with respect to high-LET radiations covering a broad range in LET and particle atomic number. Of particular interest is the establishment of experimental data for high-LET particles simulating the secondary particle spectrum following neutron exposures.

II. Benefits of the project to the European Community:

Alanine has the versatility and accuracy to replace other dosimetric systems in current use. Radiation therapy centers can use it for postal dosimetry comparisons and documentation of doses, which will benefit patients undergoing radiation therapy.
Our model, which predicts the high-LET response of alanine, has been extended to describe in detail the fading of response following high-LET irradiation. This model is essential to the use of the dosemeter in high-LET and mixed fields, and as an accident dosimeter. We have developed dosemeters with sufficient mechanical stability for these purposes.

III. Progress achieved:

1. Introduction.

The possible use of the L-α-alanine dosimetry system as an accident dosemeter has led to a series of investigations in order to develop a dosemeter which is robust, cheap to manufacture, has a broad measuring range including low doses, long term stability of response, a high reproducibility of dose measurement, and a well-characterized energy dependence of response both for high- and low-LET radiation. We have investigated the alanine dosemeter concerning mechanical and signal read-out properties, electron and photon (low-LET) dose-response characteristics, heavy charged particle and neutron (high-LET) dose-response characteristics, and predictions by model calculations of relative effectiveness and fading properties.
Alanine is suitable for use in high-LET dosimetry as the relative effectiveness, RE, decreases only by a factor of 3-4 over a LET range of five orders of magnitude, and because the dose-response maintains linearity also for the very densely ionizing particles. The response of alanine exposed to various radiation qualities can within an accuracy of a few percent be described by an exponential formula, which justifies the statistics of sensitive site activation in alanine as a single-hit detector. This makes track structure model calculations able to reproduce measured response of alanine to various radiation qualities, essentially to within experimental uncertainty (1).
We have demonstrated that at room temperature and to doses of less than 1 kGy of low-LET radiation the radical concentration is decaying slowly over a long period of time, whereas considerable decay is observed after low average doses, single tracks, from heavy charged particles (2), being strongly dependent on particle parameters, and exhibiting second order kinetics. From low-LET exposures of high dose rate to doses above saturation for radical formation (i.e. doses >500 kGy) appreciable time- and dose dependent decay of detector response was observed.
To predict decay as a function of time and particle- and detector parameters, we have set up a model (3,4) which combines two cross sections that for forming radicals, and another for decay in radical concentration in the track of the ions. The cross section for forming radicals is calculated from an exponential probability function describing measured dose-response characteristics, and the cross section for decay is calculated from a second-order polynomium fitted to describe the decay characteristics following exposures to 10-MeV electrons of high dose rate. Predictions of dose-response characteristics and fading properties after high-LET exposures are essential for the proper use of the dosemeter in radiation protection, accidental dosimetry, and in radiation therapy.
The low-LET low-dose exposures were carried out at linear radiotherapy accelerators at the University Hospital of Copenhagen, and the high-dose exposures at a linear accelerator and a ^{60}Co-γ ray source at Risø National Laboratory. The heavy charged particle exposures were carried out on Van de Graaff accelerators at the Niels Bohr Institute, University of Copenhagen, Denmark, and on the linear heavy-particle accelerator at GSI in Darmstadt, Federal Republic of Germany. The fast neutron exposures were carried out on a cyclotron at the Institute of Nuclear Physics in Cracow, Poland.

2. Properties of the dosemeter and investigation of response following low-LET exposures. .

2.1 Mechanical properties.

Due to ease of handling in transport and in use and to obtain a reasonable resolution in depth dose measurements, it was decided to prepare dosemeters in the shape of pellets with a diameter of 4.5 mm and

a thickness 2 mm. Commercially available purified crystalline amino acid L-α-alanine (for biochemistry, Merck) has been used.
Pellets compressed from pure alanine powder are brittle and not mechanically stable. This problem may be overcome by using a binder. Previously, cellulose and paraffine have been used as binders in 10 to 20% by weight. Neither of these two materials are ideal for that purpose. Cellulose contributes to the background signal of the dosemeters and paraffine has to be added in 20% by weight to obtain mechanical stability. The dosemeter added paraffine is not mechanically stable at temperatures above 50°C (2).
The heat- and mechanical stability of a dosemeter containing 5% polyvinylpyrrolidinone (polyvidone for biochemistry, Merck) was slightly better than when 10% cellulose was used as a binder and much better than when 20% paraffine addition was used. The background signals in polyvidone and in paraffine containing dosemeters were about equal and three times less than for cellulose containing dosemeters (2).
The yield of radicals produced by irradiation in pure polyvidone is about three times higher than in pure paraffine, but polyvidone is used in a concentration four times less than paraffine. The yield in pure polyvidone is only about 0.1% of the yield in pure alanine.
Further studies have shown that coating of the dosemeters with a thin layer of cellulose lacquer improves the mechanical stability considerably without affecting the dosimetric properties described.

2.2 Background signal.

The minimum detectable dose with the alanine dosemeter depends on the background signal in the dosemeter and in the ESR spectrometer. The compression of the alanine powder into pellets was found to have negligible influence on background signal. We have compared the total background signal, i.e. spectrometer- and dosemeter background, using alanine powder as received from the manufacturer and powder obtained by crushing dosemeters, that have been compressed from the same batch of alanine powder. The comparison of powder signals was necessary to obtain identical measuring situations, i.e. filling factors in the microwave cavity. No significant difference in background signal between the two powder samples could be detected.
The background signal in alanine powder was studied further by grinding and sieving of the powder. Dosemeters were prepared from this material and from alanine powder that was only sieved. The background signal was found to depend on the grain-size and being slightly higher for the larger grain-sizes. The background signal from alanine that had been ground and sieved was about two times lower than when the alanine had only been sieved. These results demonstrate, that the mechanical treatment of the alanine powder does not introduce an increased background signal.

2.3 Effect of dosemeter position in the ESR-cavity.

For the ESR measurement the dosemeter was placed horizontally on top of a suprazil quartz tube, which then by lowering positioned the dosemeter reproducibly in the cavity. For maximum reproducibility the vertical position of the dosemeter in the cavity must be reproduced within 1 mm. When the dosemeter is rotated around the vertical axis small changes in the ESR spectrum are observed with a difference in height between maximum and minimum values of about 5%. If only the height of the most prominent peak is used for dosimetry, it is necessary to reproduce the correct orientation. We have chosen to rotate the dosemeter to the position, when the height of the central peak is maximum. The alternative solution using double integration of the spectrum is also feasible but will probably not be more precise since this procedure is very sensitive to noise, and thus not useful at low doses. The ···de of the orientation effect depends on the grain-size of the alanine powder used for preparation o meter, and is 3% for grain-size b ween

56 and 160 μm and 1.6% for 50 to 56 μm. This indicates, that some alignment of the microcrystals is induced during pressing of the dosemeter.

2.4 Reproducibility of measurement.

The spectrometer is tested daily with a strong pitch sample and a dosemeter given a 10 kGy dose from a ^{60}Co-γ source.
A set of twenty dosemeters given same dose (100 Gy) of ^{60}Co-γ rays were measured and a coefficient of variation (CV) of 0.9% was obtained. When a single dosemeter was measured 10 times, i.e. removed from the ESR cavity for weighing and placed back into the cavity, a CV of 0.6% was found, which includes weighing of the dosemeter and recording of the ESR signal. Five sets of six dosemeters were each given doses of 100 Gy over a period of one year. The CV for the average dose within each set was 0.6%.

2.5 Low-dose detection limit.

For read-out purposes it was observed that the optimum value of the modulation field amplitude was 1 mT (10 Gauss) peak-to-peak and a microwave power level of approximately 10 mW.
The low-dose detection limit for practical measurements have been investigated on four ESR spectrometers (Bruker ER 300, Varian E-15, Varian E-12, and Varian E-3) and the separate effects of spectrometer- and dosemeter background, i.e. the zero-dose signal, have been determined. A set of dosemeters were given a dose of 0.3 Gy. With a single dosemeter in the ESR cavity no significant difference from background signal was observed. When five dosemeters were placed in the cavity the ESR signal was more than twice the background signal. This shows, that the background signal is dominated by the contribution from the ESR equipment. Selection of quartz tubes of highest purity and use of a clean microwave cavity is thus of utmost importance for reducing the background signal and decreasing the lower detection limit for the dosemeter. In another experiment sets of five dosemeters were given doses from 0 to 10 Gy. All five dosemeters in a set were measured simultaneously. The relation between the ESR signal and dose was a straight line with correlation coefficient r = 0.9995. A linear regression analysis gave a zero-dose signal corresponding to 0.05 Gy.
A further decrease in detection limit below 0.1 Gy thus seems possible with recent advances in ESR spectrometers, e.g. pulsed ESR spectrometers and new microwave cavities with increase in Q-values. Given these possibilities, a lower detection limit of 0.01 Gy seems realistic.

2.6 Deuterated alanine.

The prominent peaks in the ESR spectrum of the CH_3-$\dot{C}$H-COOH radical in L-α-alanine are fairly broad. Single-crystal studies (5) of irradiated CH_3-CH-(ND_3)-COOD have shown, that the deuteration leads to narrower lines. If similar effects occur in irradiated powders a significant increase in signal height would result, since the signal height is proportional to (number of radicals)/(line width)$^{1/2}$.
We have prepared dosemeters from CH_3-CH-(ND_3)-COOD as well as from CD_3-CD-(NH_3)-COOH and exposed them to ^{60}Co-γ rays. All dosemeters were given same dose. Spectra were obtained at low modulation amplitude to avoid obscuring fine structure. It was found, that the line width is slightly smaller in CH_3-$\dot{C}$H-COOD than in CH3-$\dot{C}$H-COOH, but this only results in better resolution of the fine-structure. CD_3-CD-(NH_3)-COOH only shows a single line from CD_3-$\dot{C}$D-COOH with a high degree of fine-structure. It is evident from the results obtained, that no increase in sensitivity is possible with deuterated dosemeters.

2.7 Dose-response as a function of energy.

The change in effectiveness with photon energy has previously been studied only for energies below that of ^{60}Co-γ rays.
We have used beams of 6- to 20-MeV electrons and 4- to 22-MV photons produced by linear accelerators. Dosemeters were exposed in sets of six at a time to doses from 25 to 100 Gy. The response was compared with response of dosemeters exposed to ^{60}Co-γ rays. The calibration of both linear accelerators and ^{60}Co-γ ray source was specified as dose to water. To convert the specified photon doses to water into dose to alanine, the energy absorption coefficient for alanine relative to water was calculated using the data of Hubbel (6). Also for the electron beams, the electron collision stopping power has been calculated for alanine relative to water from the formula given by Seltzer and Berger (7). It was found that the employed method is fully satisfactory in correcting for the difference between alanine and water, which makes the dosemeter eminently suited for calibration comparisons between radiation therapy centers.
A comparison involving centers in Poland, Finland, USA, and Denmark has already been carried out with encouraging results. Some of the results have been presented at international scientific meetings (8,9) and attracted considerable interest in the possibilities of the dosemeter in intercomparison studies.

2.8 Stability of low-LET radiation-induced radicals.

For dosemeters exposed to doses below 1 kGy no significant change (<1%) is seen over a period of one year. Dosemeters were given 10-100 Gy from either ^{60}Co-γ rays or 4- to 16-MV photons and 6- to 20-MeV electrons and kept under normal laboratory conditions. The dosemeters exposed to ^{60}Co-γ rays showed a fading of less than 2% over a period of 4-1/2 year, while the linear accelerator-irradiated dosemeters exposed with a much higher dose rate showed a change of 4-5%. These results demonstrate the excellent stability of the dosemeter and its value as an integrating dosemeter over a very long period of time, which is important in accident dosimetry.

3. Measurement and prediction of relative effectiveness and fading characteristics following high-LET exposures.

3.1 Dosimetry.

The investigated particles are all but the 16-MeV protons completely stopped in the dosimeter, and the absorbed dose was calculated from kinetic energy of the particle, particle fluence,and stopping power data (10). The relative effectiveness is calculated from the expression:

$$RE = R_g \times S_i / (\pi/4 \times d^2 \times E \times A \times \Phi) \qquad (1)$$

where R_g is the change in response per unit mass and per unit dose of gamma-rays. The change in response per unit mass of exposed alanine and per unit of heavy particle dose is calcilated from S_i the change in response due to ion exposure, d the diameter of the detector perpendicular to the beam direction, E the kinetic energy of the particle, A the particle atomic number, and Φ the particle fluence.
The fast neutrons of 5.6-MeV mean energy were produced via the ^{10}Bc(d,n)^{9}B-reaction from 12.5-MeV deuterons bombarding a beryllium target. The gamma contamination of the total dose was 4%. Exposure was converted into absorbed dose in water (11). A Kerma factor of 0.86 was obtained from tables of Bach and Caswell (12). The total beam dose, $D_T = D_n + D_g$, consists of neutron (D_n) and gamma (D_g)

components, where $D_n = 0.96 \times D_T$ and $D_g = 0.04 \times D_T$. Assuming additivity of the alanine responses due to the neutron ($S(D_n)$) and gamma ($S(D_g)$) contributions, the relative effectiveness of pure neutrons can then be calculated from the expression:

$$RE_n = ((R_T/R_g) - 0.04)/(0.86 \times 0.96) \quad (2)$$

where R_T is the signal per unit of total beam dose, R_g the signal per unit of gamma-ray dose.

3.2 Dose-response and relative effectiveness.

Dose-response characteristics have been measured for the following high-LET radiation qualities: 1-,2-,3-, 6-, and 16-MeV protons, 4-MeV/u ^{16}O-, 18.5-MeV/u ^{40}Ar-, 15.2-MeV/u ^{40}Ca-, 15.4-MeV/u ^{58}Ni-, 13.8-MeV/u ^{208}Pb-, 5.9- and 15-MeV/u ^{238}U-ions covering a range in initial LET of 68-166,000 MeVcm^2/g, and to fast neutrons of 5.6-MeV in average energy. From measured dose-response characteristics and derived radiation sensitivities experimentally obtained values of RE are shown in table 1 together with theoretical values obtained from model-calculations (1,13).

The rate with which the RE decreases with LET depends on the radiation sensitivity of the detector and particle parameters as charge and velocity. Alanine is relatively insensitive to ionizing radiation and therefor RE decreases only slowly with LET, being 1 for the 16-MeV protons with LET of 27 MeVcm^{2}g^{-1} and approximately 0.3 for the 5.9-MeV/u uranium ions with LET of 1.66×10^5 MeVcm^{2}g^{-1}, i.e. a factor of three to four in RE over five orders of magnitude in LET. For particles of the same velocity and being stopped in the detector, the RE will be a decreasing function of LET, e.g. comparing the 15.4-MeV/u ^{58}Ni-ions with the 15-MeV/u ^{238}U-ions. For particles of the same atomic number the slowest one will always show the lowest RE, e.g. comparing the 15-MeV/u and the 5.9-MeV/u ^{238}U-ion. The RE-LET relationship in the Bragg-peak of a stopping particle is more complex (14). For different kinds of ions of different velocity only calculations will be able to predict the RE, e.g. the 4-MeV/u ^{16}O-ions and the 18.5-MeV/u ^{40}Ar-ions. For comparison we have shown in table 1 calculated values of RE based on our track structure model, and it is observed that these data very well predict the relationship between RE and particle atomic number and velocity.

To emphasize the impracticability of expressing the relative effectiveness of heavy ions as function of LET (15) we have compared our predicted data (16) with data of Henriksen (17), who has measured RE for different ions of the same velocity bombarding an alanine detector. This comparison shows, a) that LET is not a sufficient parameter in describing RE of a detector, and b) that our model yields predictions of RE in fair agreement with Henriksen's data.

A brief comparison of the ESR spectra following high-LET exposures has shown only small but significant differences. The typical 5-line spectrum of alanine shows the main peaks to be overlapped by another line from a second and stable radical, the formation of which relative to the prominent radical depends on particle parameters. The ratio A/B, table 1, shows the peak height from the prominent radical (A) relative to the peak height from the second radical (B), indicating the relationship between the two radicals for the investigated particle parameters. The pellets were placed in the ESR cavity such that maximum signal was obtained. These observations indicate, that the high doses in the track of the high-LET particles mainly reduce the second radical, which then could be the reason for an observed decrease in saturation response for increasing LET (18). However, a detailed investigation of spectral changes should be made with single crystals,as the shape of the spectrum depends on the orientation of the crystal in the ESR cavity.

3.3 Fading of response.

Decay in radical concentration, fading, in alanine has been studied at room temperature for low average doses of the following radiation qualities: ^{60}Co-γ rays, 10-MeV electrons, 1-, 2-, 3-, 6-, and 16-MeV protons, 4-MeV/u ^{16}O-, 15.2-MeV/u ^{40}Ca-, 15.4-MeV/u ^{58}Ni-, 13.8-MeV/u ^{208}Pb-, and 5.9 and 16.5-MeV/u ^{238}U-ions covering a range in initial LET of 2-166,000 MeV cm2/g, and from 5.6-MeV neutrons. Fading was expressed in per cent as relative change in ESR signal as a function of time. Table 1 shows fading of response 1000 hours after exposure.

Fading characteristics as a function of time following exposures to very high doses have been obtained for 10-MeV electrons of high dose rate and ^{60}Co-γ rays, and it turns out that the amount of fading increases strongly with dose and dose rate.

For high average doses ($>10^5$ Gy) from particles with Z> 20 used in this work and from the 5.6-MeV neutrons a time dependent increase in response of 5-10% has been observed reaching a maximum approximately 100 hours after exposure. Thereafter a decay takes place but with a less steep slope than observed for exposures to lower doses. This increase in response must be due to induced radioactivity in the detector. As the heavy particles penetrate only a few hundred microns into the surface of the detector, the remaining and unirradiated part will be exposed to the gamma rays emitted from the radioactive fission processes. Exposures with low energy protons to the same doses do not show any increase in response.

3.4 Prediction of fading.

On basis of our observations at low average doses, we consider that the difference in fading of response between low- and high-LET radiation is ascribed to radical recombination effects in the high-dose regions of the heavy charged particle tracks, and should be predicted from the structure of the particle track, detector parameters, and measured decay characteristics following low-LET exposures of high dose rate.

The radial dose distribution is calculated as an average dose deposited in the radiation-sensitive element of the detector, which is considered to be a sphere, the radius (a_o) of which is derived from target theory (13,19).

The radial dose distribution is calculated from:

$$D(z,\beta,t) = k_1 \times \frac{z^2}{\beta^2} \times \frac{1}{\alpha} \int_{t-a_o}^{t+a_o} \frac{1}{t} \times \left(1 - k_2 \times \left(\frac{1-\beta^2}{\beta^2}\right)^{\alpha}\right) \times A(t,a_o)\, dt \quad [Gy] \qquad (3)$$

where z is the effective charge of the ion, β is the ion velocity in terms of the velocity of light, k_1, k_2, and α are constants, t is the distance from the ion path, and $A(t,a_o)$ is a geometric parameter.

The experimentally obtained dose-response characteristic for alanine exposed to ^{60}Co-γ rays or 10-MeV electrons (1,2) shows that saturation sets in at doses above 10^4 Gy. This dose-response characteristic can be expressed by an exponential function describing the probability of creating radicals as a function of dose:

$$P(D) = 1 - \exp(-D/D_o) \quad [\text{relative units}] \qquad (4)$$

Here D is the dose and $D_o = 1.05 \times 10^5$ Gy is a characteristic dose for L-α-alanine corresponding to the dose at which 63% of the saturation response is obtained.

A second-order polynomial fitted to the experimentally obtained time dependent decay characteristics following exposures to 10-MeV electrons yields a formula describing the relative decay as a function of dose D[Gy] and time T[hours] after exposure:

$$R(D,T) = (a + b \times D + c \times D^2) \times (d \times \ln(T) - 1) \quad [\text{relative units}] \qquad (5)$$

where $a=3.031\times10^{-3}$, $b=9.312\times10^{-9}$, $c=3.958\times10^{-15}$, and $d=1.443$ are detector-dependent constants.
The cross section for radical formation in the track of a heavy particle is calculated from the probability of creating radicals as function of dose (eq.4) and the radial dose distribution (eq.3):

$$\sigma_p(z,\beta) = 2\pi \int_0^{t_{max}} P(D(z,\beta,t))\times t \; dt \quad [cm^2] \tag{6}$$

where t_{max} is the range of the secondary electrons generated by elastic collisions between the ion and the atomic electrons of the target.
A cross section for post-irradiation recombination of radicals as a function of particle- and detector parameters and time can now be derived from radial dose distribution (eq.3), radical decay (eq.5), and from cross section for radical formation (eq.6):

$$\sigma_r(z,\beta,\sigma_p,T) = \sigma_p(z,\beta)\times\frac{1}{t_{max}} \int_0^{t_{max}} R(T,D(z,\beta,t)) \; dt \quad [cm^2] \tag{7}$$

The predicted radical decay kinetics for the ions investigated in this work have been compared with the experimental data. Although no full agreement exists between calculated and measured data, particularly for the very heavy particles, the overall trend in the result is acceptable indicating the potential of the model, but also that improvements are necessary. In the calculations spallation and fission products of the ions are not taken into consideration, thus being a possible explanation for the discrepancy at particles with $Z > 20$. The uncertainty, however, in the experimental data shown here is rather high and is due to the nature of handling two essentially equal numbers to obtain the change in response.
The experimental data show that even though the calcium ions have a much higher stopping power than have the oxygen ions, the decay is less for the calcium ions.This phenomenon is related to the lower initial energy of the oxygen ions for which a relatively larger amount of energy is deposited in the track at doses above saturation for radical production, and parallels that obtained for the relative effectiveness. The predicted decay for these two ions show the same effect emphasizing the capability of the model in reflecting the specific particle parameters.

4. Conclusion.

We have demonstrated the applicability of the alanine detector in the dosimetry of γ ray-, electron-, heavy ion-, and neutron beams, and we have demonstrated an accuracy of about 1-2% in determination of doses from low-LET radiation in the dose range of 50-5000 Gy. We have further shown the suitability of the alanine detector for calibration- and intercomparison dosimetry, and for accidental- and personal dosimetry even in mixed fields. The alanine detector is well suited in radiotherapy, as it can be used for documentation of doses to patients and for postal calibration of radiotherapy radiation units due to its long term stability of response.
We have demonstrated the ability of our model to predict relative effectiveness and radical decay kinetics following heavy charged particle exposures of the alanine dosemeter over a broad range in LET. However, as the work on decay kinetics is still in progress the data presented here should be considered as preliminary.
In order for a dosimetric system to be applicable in practice, an accompanying model is required which can analyze and predict the response of this system to radiations of all qualities. Track-structure theory which relates the signal of a detector after doses of heavy charged particles with its signal after doses of gamma-rays or fast electrons, is able to fulfil these requirements.
Together with model calculations the alanine detector could constitute a system for absolute dosimetry highly competitive with the presently used Fricke ferrous sulphate dosemeter, especially as a tissue-equivalent detector.

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Table 1.

		LET_{init} $MeVcm^2g^{-1}$	RE measured	RE calculated	Decay measured %	Decay calculated %	Ratio A/B
16.0 MeV	protons	27	1.00±o.o3	0.98	3.0±0.5	2.7	1.35
6.0 MeV	protons	68	0.86±0.03	0.85	3.2±1.0	3.0	1.35
3.0 MeV	protons	117	0.74±0.07	0.77	4.5±0.6	3.2	1.51
2.0 MeV	protons	159	0.65±0.02	0.70	4.2±0.2	3.4	1.70
1.0 MeV	protons	259	0.54±0.04	0.58	5.1±0.7	3.7	1.64
4.0 MeV/u	^{16}O-ions	5.27 10^3	0.32±0.02	0.31	8.1±1.7	6.9	1.41
18.5 MeV/u	^{40}Ar-ions	7.86 10^3	0.37±0.02	0.36			1.55
15.2 MeV/u	^{40}Ca-ions	1.14 10^4		0.34	6.2±0.8	6.0	1.90
15.4 MeV/u	^{58}Ni-ions	2.32 10^4	0.35±0.07	0.28	6.4±1.2	4.9	1.63
16.5 MeV/u	^{238}U-ions	1.14 10^5		0.19	6.3±1.7	3.7	1.76
13.8 MeV/u	^{208}Pb-ions	1.25 10^5	0.25±0.05	0.23	6.7±0.9	3.7	1.56
15.0 MeV/u	^{238}U-ions	1.35 10^5	0.33±0.06	0.19			1.74
5.9 MeV/u	^{238}U-ions	1.66 10^5	0.27±0.03	0.19	8.9±0.8	4.1	1.67
5.6 MeV	neutrons		0.62±0.03				1.40

Errors indicate one standard deviation.

IV. Other research group collaborating actively on this project:

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1.

Hansen, J.W., Olsen, K.J., Wille, M., Radiat.Prot.Dos. Vol.19 No.1, 43-47 (1987).
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RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

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National Physical Laboratory
Queens Road
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Contract no.: BI6-A-003-UK

Head(s) of research team(s) [name(s) and address(es)]:

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Title of the research contract:

Development and investigation of a reproducible multisphere system for radiation protection purposes, and its use to correct personnel dosemeter measurements.

List of projects:

1. Development and investigation of a reproducible multisphere system for radiation protection purposes, and its use to correct personnel dosemeter measurements.

Title of the project no.:

The design, construction and calibration of a multisphere spectrometer system for radiation protection purposes; and its use to correct personnel dosemeter measurements.

Head(s) of project:

Dr J B Hunt

Scientific staff:

Dr D J Thomas and Mr A G Bardell

I. Objectives of the project:

The main objective is to provide a specification for the contruction of a reproducible multisphere spectrometry system for use in stray neutron fields. Through a combination of computational techniques and measurements in standardised neutron fields, both monoenergetic and broad-range, a set of response functions appropriate to the specification will be determined. Where possible, recommendations and simple recipes for the assessment of neutron spectra and/or neutron dose equivalent will be provided. It is also hoped to use the system to compute correction factors for typical dosemeters employed at specific locations.

II. Objectives for the reporting period:

During this period it was hoped to complete the analysis of the calibration data obtained earlier, to calibrate the multisphere system for incident thermal neutrons, and to obtain integral responses for well-characterised radionuclide neutron sources. It was hoped to carry out some measurements under field conditions. On the computational side, to use the discrete ordinates neutron transport code ANISN to calculate the response matrix, and to investigate the effect of small changes in the input data, particularly arising from variations in polyethylene density. It was planned to test the adopted unfolding code, STAY'SL, through participation in a EURADOS Committee IV organised benchmark intercomparison.

III. Progress achieved:

In order to derive a neutron spectrum using a multisphere set a necessary pre-requisite is that the response as a function of energy must be known for each sphere. Although it is possible to calculate the response functions at neutron energies from thermal up to 20 MeV, the reliability of the calculations is uncertain, particularly at the higher energies, and so calibrations employing incident monoenergetic neutrons are highly desirable. Unfortunately, such calibrations can only cover a small part of the required energy range. Thus, the adopted programme of work makes use of both approaches in order to derive the optimum response function for each sphere.

The NPL sphere set has been calibrated for thermal neutrons and at twelve further energies from 1.2 keV to 14.8 MeV. Corrections have been applied for dead-time effects, room and air inscatter contributions, air attenuation or outscatter, noise, photon contamination, and neutron scattering by the spheres, neutron fluence devices and the monitors into each other. The analyses of all the data have been completed except for a small correction to account for low energy neutrons scattered within the target assembly itself. This correction has now been evaluated for neutron energies between 144 keV and 1.2 MeV, and work is in progress to derive correction factors for each sphere at each of the other neutron energies.

Analysis of the thermal measurements performed on the bare ^{3}He proportional counter, and on the smaller spheres, is now complete. In order to calculate each sphere response as a function of neutron energy, the number density of ^{3}He nuclei in the proportional counter must be known. This can be derived from a measurement of the response of the bare detector in a known thermal neutron fluence. An exact expression for the response of a spherical detector has been derived and compared with a number of approximate formulations used in earlier work. Due allowance has also been made for the effective temperature of the Maxwellian distribution, for the epithermal component of the calibration field, the variation of response of the detector as a function of energy, and the effects of absorption and scattering within the spherical steel shell of the counter and the krypton component of the counting gas mixture. A detailed report of this work (NPL Report RS(EXT)104) has been published.

The neutron transport code ANISN has been used to calculate the response of the bare detector and all the spheres over the energy range from thermal to 14 MeV. Calculations have also been performed to predict the change of response as a function of both the ^{3}He number density within the central detector and the density of polyethylene. The responses are

found to vary significantly with the latter, and corrections have to be applied if there are density variations over the sphere set. Preliminary comparisons of the calculated responses with the measurements reveal reasonable agreement at energies in the keV and MeV region, but suprisingly poor agreement for the small spheres at thermal energies. The reason for this is not understood at the present time.

Preliminary work has started on the optimisation of the response matrix, taking into account both the ANISN calculated results and the measured response values. The unfolding program STAY'SL is being used in a novel way for this part of the project. For each sphere, the measured response values and the associated neutron spectra are input as fixed values with associated uncertainties and correlations. The ANISN calculated response as a function of energy is then used as an initial solution, and adjusted in in order to minimise the difference between the fitted response and the measured response. Such a technique takes into account all the uncertainties and correlations, and changes in the shape as well as a simple normalisation are possible. The initial results obtained are extremely encouraging.

The use of multispheres for spectroscopy requires a computer code to unfold the neutron spectrum from the measured count-rates. The unfolding code STAY'SL has been adopted for this task because it properly takes into account all the uncertainties and the associated correlations. In a recent benchmark unfolding intercomparison organised by EURADOS Committee IV, the results obtained by NPL, using STAY'SL were among the best reported.

Field measurements have been made at a number of different sites in two nuclear power stations, and the analysis of these data is in hand. Measurements were also made using conventional area survey meters.

IV. Objectives for the next reporting period:

Measurement programme: The analysis of all the earlier measurements will be completed, and further calibrations using thermal neutrons and radionuclide neutron sources will be undertaken. It is hoped to carry out further measurements under field conditions.
Computational programme: The final response matrix will be evaluated, taking all the measurements and the calculations into account. Correction factors to take into account variations in the ^{3}He number density and polyethylene density will be finalised. Participation in EURADOS Committee 4 benchmark intercomparisons will continue.

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and some members of EURADOS Committee 4, "Numerical Dosimetry".

VI. Publications:

Alevra A V, Cosack M, Hunt J B, Thomas D J and Schraube H O E.
Experimental determination of the response of four Bonner sphere sets to monoenergetic neutrons.
Radiat. Prot. Dosim., 23, 293-296, (1988).

Thomas D J and Soochak N.
Determination of the ^{3}He number density for the proportional counter used in the NPL Bonner sphere system.
NPL Report RS(EXT) 104, July 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
GSF
Ingolstädter Landstr. 1
D-8042 Neuherberg b.München

Contract no.: B16-A-172-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. W. Jacobi
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GSF
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Dr. G. Burger
Institut für Strahlenschutz
GSF
Ingolstädter Landstr. 1
D-8042 Neuherberg b. München

Telephone number: 89-31.87.22.25

Title of the research contract:

Radiation exposure analysis and biological dosimetry.

List of projects:

1. Exposure analysis for occupationally, medically and accidentally exposed persons (with emphasis on neutron irradiation).
2. Microdosimetry and biological dosimetry.

Title of the project no.:

Exposure Analysis for Occupationally, Medically and Accidentally Exposed Persons (with Emphasis on Neutron Irradiation)

Head(s) of project:

A. Wittmann

Scientific staff:

A. Wittmann, E. Mannweiler,
G. Burger

I. Objectives of the project:

Due to structural changes in the group and unforeseen personnel problems the objectives of project no. 1 had to be revised. The work is restricted now to theoretical-numerical investigations on quantities being of relevance in neutron radiation protection, and their revisions with respect to recently discussed assumptions on new quality and risk factors. Working place and exposure analysis is no longer performed by the contractors (G.B.) research team.

II. Objectives for the reporting period:

1. Continuation of phantom improvements based on patients' organ reconstructions and numerical radiation transport methods.
2. Investigations on new quality and risk factors and their impact on neutron conversion functions being at relevance in radiation protection.

III. Progress achieved:

1. The software for generation of voxel based 3D-phantoms from series of tomographic images gained by CT or MR-tomography was further extended and improved. Image processing procedures were investigated necessary for contrast enhancement as a prerequisite of organ segmentation especially in the lower quality fast MR-3D sequences. In order to overcome the time consuming MC-calculations and the shortcomings of the CHORD-method for the calculation of organ doses, the pencil beam method based on the superposition of scatter dose distributions of ideal narrow beams (rays) was further investigated. The main problems occur in non-homogeneous phantoms with clearly different neutron scatter cross sections in adjacent tissues.

2. The current discussion on new definitions of the quality factor for charged particles (ICRU 40) was continued by additional proposals of Kellerer and Hahn (IMSK 88/119, priv. comm.) for Q(L) and Q(y). For these proposals the quality factor for neutrons were calculated, taking into account the protons only for the time being. Fig. 1 shows the result for the three proposed Q(L)-models. The maximum which is well represented by the protons only is in between the results for ICRP 51 and ICRU 40 recommendations. The deviations of the Q(y) approach to the one presented in ICRU 40 are negligible.

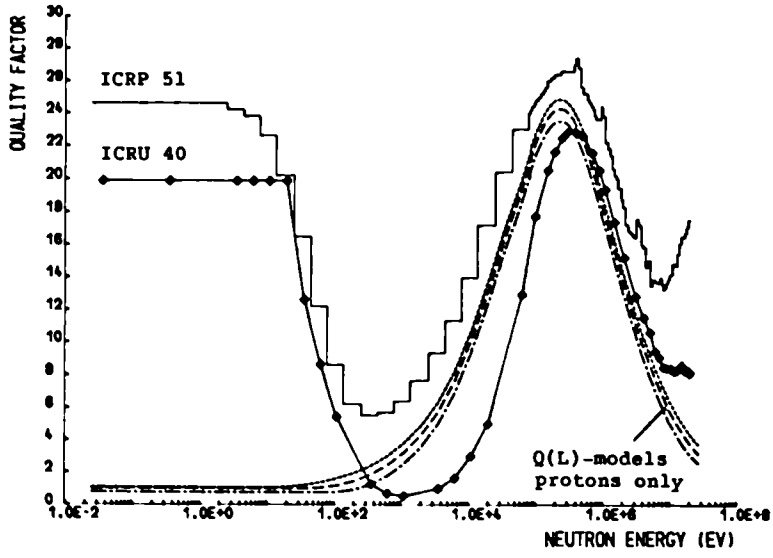

Fig. 1

Finally new risk factors are discussed based on recent A-bomb survivor analysis. They result in revised weighting factors for the effective dose equivalent H_e (see Table I). The influence of the revision on H_e for four different exposure geometries was assessed by using the old quality factor (ICRU 16). Due to the high weighting factor for the remainder, the problem of an unequivocal definition of it becomes again important. We used model 5 in Burger et al. IV Symp. on Neutron Dosimetry, p41, 1981. Fig. 2 shows the ratio of H_e for both sets of weighting factors. The results differ by 15 % at maximum, especially in the intermediate energy region where the greatest differences between organs are occuring due to the steep depth dose curves.

Table I: New proposal for organ risk factors and weighting factors for H_e.

Organ	Risk Factors (10^{-4}/Sv) male	female	Weighting Factors
Lung	40	40	0.08
Testes	80	0	0.08
Thyroid	6	14	0.02
Breast	0	150	0.15
Ovaries	0	80	0.08
RBM	40	40	0.08
Remainder	250	250	0.5
Bone Surface	5	5	0.01

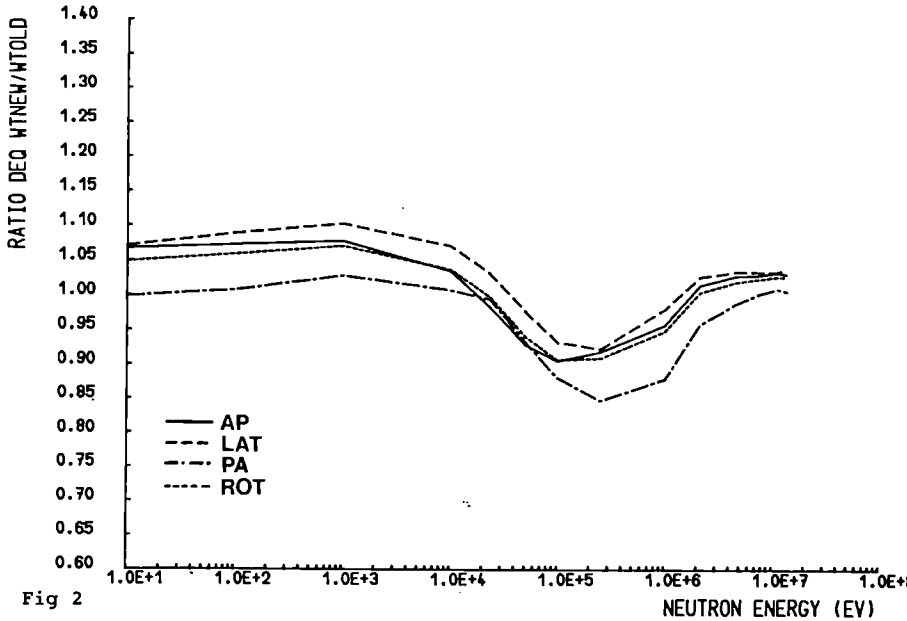

Fig 2

IV. Objectives for the next reporting period:

The first project will be terminated by completion of neutron conversion functions, and compilation and evaluation of existing data on radiation fields in working environments. Emphasis will be shifted continuously for the remaining contract period on to project no. 2.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Drexler, GSF-Neuherberg

Dr. Siebert, PTB-Braunschweig

VI. Publications:

Title of the project no.:

Microdosimetry and Biological Dosimetry

Head(s) of project:

G. Burger

Scientific staff:

G. Burger, G. Leuthold, M. Aubele,
M. Heymann, A. Chaudhuri, U. Jütting

I. Objectives of the project:

The objective of the project is an improved understanding of biological efficiency of radiation. This necessitates as a first step a thorough analysis of the physical stages of radiation interaction and local energy deposition in model targets of living matter. The investigations include calculations of energy distributions in cavities, the continuation and extension of charged particle track structure analysis and studies on cytometric biological dosimetry.

II. Objectives for the reporting period:

1. Extension of track simulation calculations and energy deposition analysis in small cavities and application to secondaries in case of photon and neutron irradiation of phantoms.
2. Improvements and application of the NBS-Washington code for proportional counter calculations in collaboration with several groups within EURADOS.
3. Continuation of investigation of cytometric assays for biological dosimetry.

III. Progress achieved:

1. The lineal energy $\overline{y}_D$ is a well established quantity not only in analytical radiobiology but even used in recent recommendations on quality factors for neutrons (ICRU 40). This implies assumptions on target sizes. For targets in the micrometer diameter region $\overline{y}_D$ may be calculated by using the LET-approximation for the charged secondaries as in the programme of J. Coyne, for smaller target sizes which may be of higher radiobiological relevance the detailed track structure has to be taken into account. Based upon track structure calculations for protons and α-particles (and neglecting the role of heavy recoils for the time being) $\overline{y}_D$ was determined as a function of neutron energy in the energy interval between 0.036 eV and 14.1 MeV for target diameters below 100 nm. Fig. 1 shows $\overline{y}_D$ for 1, 10 and 100 nm target diameter together with those for the LET-approximation of a 1 micron target diameter by Coyne et al.

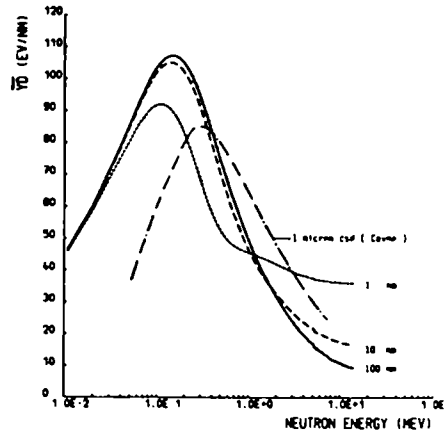

Fig. 1

The energy deposition by simulated α-particle tracks transversing spherical targets of 50 to 400 nm diameter was calculated for 0.25 to 1 Mev/amu α-particles in collaboration with the University of Padua. At the smallest target size straggling effects lead to clearly asymmetric distributions.

3. Biological dosimetry

Due to promising results in the pilot studies using white blood cells from whole body irradiated mice rather than spermatozoa some emphasis was layed upon methological aspects of sampling and preparation for improving the cell blood assay. As a result it became quite clear e.g. that no conventional blood smears can be used but only monodisperse cytospin specimens. An important investigation concerned the kinetics of morphological expression of the radiation effect on lymphocytes as a function of time after exposure. Table I shows the results of a linear

discriminant analysis using 25 mice whole-body irradiated with 2 Gy, grouped into five mice each sampled at five different days after exposure and a control group. From each mouse 100 lymphocytes are measured by means of high resolution image analysis. The cytometric changes are most pronounced after 7 to 14 days as demonstrated by the distribution of the pooled cell populations in the feature space. In Fig. 2 the distances of the centers of each pool for the treated mice to the control group are plotted.

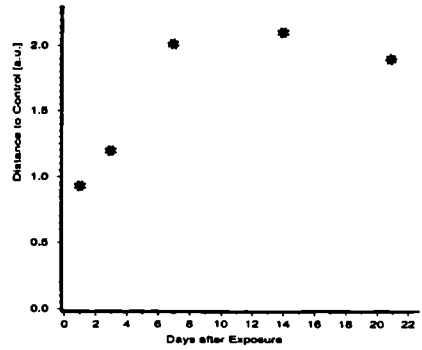

Fig. 2

Table I: 6-class discrimination analysis of lymphocytes from the control group and irradiated mice measured after 1, 3, 7, 14, and 21 days after exposure

	%correct	Control	2Gy 1d	2Gy 3d	2Gy 7d	2Gy 14d	2Gy 21d
Control	42.2	422	172	117	41	44	118
2Gy 1d	44.2	114	221	46	57	11	51
2Gy 3d	27.0	107	42	134	97	66	53
2Gy 7d	63.8	33	54	72	319	10	12
2Gy 14d	64.7	16	13	49	9	325	90
2Gy 21d	66.1	22	35	36	18	59	331
total	53.1 %						

These investigations were accompanied by an independent experiment on 30 mice undergoing experimental tumor transplantation. White blood cells as well as squamous epithelial cells in vaginal smears were investigated to follow the history of tumorigenesis by the malignancy associated change-assay in non involved tissues. The results of a pilot study (5 mice in each group) in distinguishing tumor positive and negative mice showed about 85 % correct single cell classification.

The idea of this subproject is to define sensitive cytometric endpoints for in vivo assays of radiation induced acute as well as late somatic effects.

IV. Objectives for the next reporting period:

1) Completion of $\overline{Y}_D$-calculations for target sizes down to 10 nm for neutrons and gammas in the whole energy region of interest in radiation protection and application to phantom calculations.
2) Performance of dose effect studies for mouse sperms and leucocytes for gammmas and eventually fission neutrons.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Siebert, PTB Braunschweig

Dr. Colautti, INFN, Legnaro,

Dr. Kummermehr, GSF, Neuherberg

Dr. Guttenberger, GSF, Neuherberg

Dr. Kellerer, Univ. Würzburg

VI. Publications:

Leuthold, G., Burger, G. : Mathematical simulation of proton tracks in water vapour and their microdosimetric analysis. Radiat. Environ. Biophys. 27, 177-187 (1988)

Leuthold, G., Burger, G.: Dose mean lineal energy for fast neutrons in small spherical targets. Radiation Protection Dosimetry 23/1. Kent, England: Nuclear Technology Publishing, 49-51 (1988)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
GSF
Ingolstädter Landstr. 1
D-8042 Neuherberg b.München

Contract no.: BI6-A-011-D

Head(s) of research team(s) [name(s) and address(es)]:

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Dr. H.G. Paretzke
Institut für Strahlenschutz
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Ingolstädter Landstr. 1
D-8042 Neuherberg b. München

Telephone number: 89-31.87.22.25

Title of the research contract:

Track structure calculations.

List of projects:

1. Track structure calculations for radiation risk estimation.

Title of the project no.: 1

Track structure calculations for radiation risk estimation

Head(s) of project:

H.G. Paretzke

Scientific staff:

K. Long, S. Henß, H.G. Paretzke

I. Objectives of the project:

Track structures of charged particles describing the primary locations of relevant molecular changes produced by irradiation will be calculated for complex, heterogeneous, condensed targets (cell, etc.) and evaluated with respect to the characteristics determining their biological consequences (e.g. DNA damage, cellular damage). The radiation fields considered include internal emitters, external photon fields and HZE-particles. To this purpose the existing Monte Carlo-Computer programs for particle transport simulation will be modified with respect to the physical cross section data base, the geometrical scoring routines and the methods of classification of results.

II. Objectives for the reporting period:

- Evaluation and implementation of proton and alpha particle inelastic scattering cross sections for the same 15 target materials from 1 keV to 1 GeV per nucleon.
- Improvement of the local density functional theory and application to other target molecules (DNA and -bases); derivation of electron inelastic scattering cross sections from 1 eV to 100 MeV for condensed biological target molecules.

III. Progress achieved:

a) Methodology:

- The energy differential secondary electron ejection cross sections for 15 target materials (including liquid water, DNA, and polymers and gases used in experimental microdosimetry) and the projectiles proton and alpha-particles have been calculated approximately by the empirical method proposed by M.E. Rudd (Phys. Rev. A38, 6129-6137, 1988); classical assumptions were made regarding the angular distributions.
- Double differential electron impact cross sections for ionization were calculated using the first approximation to time dependent density functional theory based on a set of contracted Gaussian-type orbitals within the Hartree-Fock approximation.
- The charged particle track structure codes were applied to calculate characteristic quantities of radiation biological importance.

b) Results:

- The energy differential ionization cross sections for protons and completely stripped alphaparticles were calculated using the empirical approach of Rudd; the target materials considered are water, H_2, He, Ar, O_2, and polymeric biomolecules. These energy dependent cross section will now be used in the ion-track structure code MOCA-15.

- The density functional theory was used to calculate double differential electron ejection cross sections for electron impact on a number of target molecules. In the primary energy range above 200 eV the differences due to phase status are very small (see fig. 1)

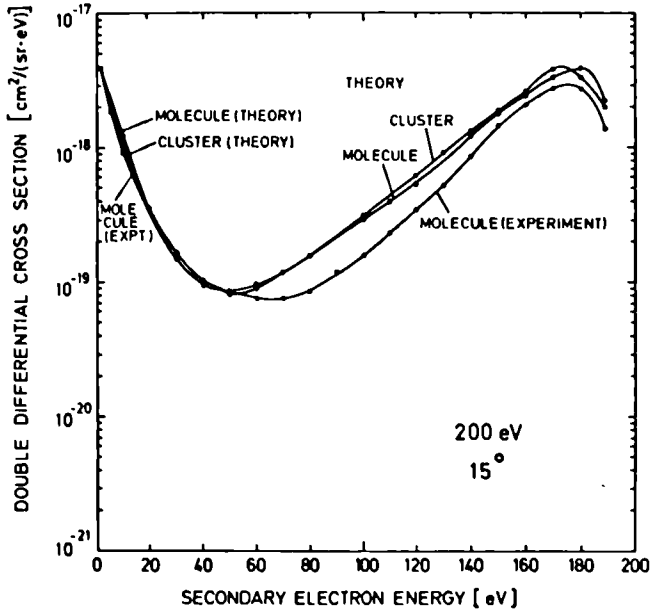
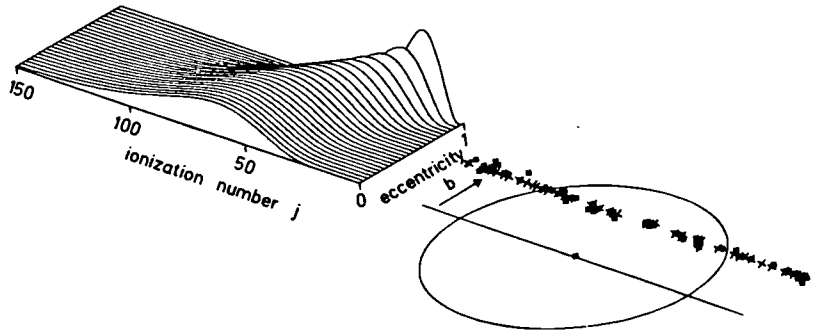


Fig.1 Double differential cross section for 200 eV electrons on water molecule (Exp.+Theory) and central molecule of cluster (Theory)

- For various primary charged particles the statistical density distributions for energy deposited and number of ionizations produced in small target volumes as a function of eccentricity were calculated (fig. 2). It was found that close to the surface of a target



the LET-approximation could lead to wrong estimates of these distributions.

c) Discussion:
- The proton cross sections appear to agree reasonably well with experimental data. For slow alphaparticles carrying one own electron the existing derivation of predicted and experimental values makes improvements necessary by consideration of charge-transfer processes into the continuum.
- The quantum chemical calculations need to be extended into lower primary particle energies where larger phase differences are expected.
- A feasable approach for characterization of charged particle tracks as regards their radiobiological effectiveness by more than one quantity has to be developed; apparently combined effects on three different structural levels are determining this effectiveness.

IV. Objectives for the next reporting period:

a) The work on all interaction cross-sections needed in this project will be finalized and described taking shortcomings into account which cannot be resolved presently (due to the lack of adequate experiments and theories).
b) The HZE-cross sections will be extended to the parameter range used in radiobiological experiments at GSI and Bevelac for the interpretation of the experimental data obtained there.
c) The alphaparticle and electron code will be used to estimate the potential hazard from incorporated "warm" or "hot" particles emitted from the Chernobyl reactor.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Physical aspects: Dr. Booz (KFA Jülich) and other members of EURADOS-committee 1, Prof. Blanc (Univ. Toulouse), Prof. Sigmund (Univ. Odense), Prof. Hatano (Inst. of Technology, Tokyo), Dr. Toburen (Battelle, Richland), Dr. Inokuti (Argonne National Laboratory, USA), Dr. Turner (Oak Ridge National Laboratory), Dr. Diercksen (Max-Planck-Institut, Garching).

Biological aspects: Drs. Dennis, Lloyd (NRPB, Chilton), Drs. Broerse, Barendsen (TNO, Rijswijk), Dr. Goodhead, (MRC, Chilton), Dr. Lafuma (CEA, Fontenay-aux-Roses), Prof. Hall (Columbia Univ., N.Y.)

VI. Publications:

Wilson, W.E., Paretzke, H.G.:
An Analytical Model for Ionization Distributions Produced in Nanometer Volumes by Recoil Protons.
Rad. Prot. Dos. 23, 45-48 (1988)

Wilson, W.E., Metting, N.F., Paretzke, H.G.:
Microdosimetric Aspects of 0.3 to 20 MeV Proton Tracks, I. Crossers.
Radiat. Res. 115, 389-402 (1988)

Paretzke, H.G.:
Problems in Theoretical Track Structure Research for Heavy Charged Particles.
in: Quantitative Mathematical Models in Radiation Biologie, J. Kiefer (Ed.), Springer-Verlag, Heidelberg, S. 49-56 (1989)

Paretzke, H.G.:
Energy Deposition at the Molecular and Cellular Levels.
In: Mechanisms of Radiation Interaction with DNA: Potential Implication for Radiation Protection, U.S. Dept. of Energy, Nat. Techn. Inform. Sevice, Springfield, 39-47 (1988)

Paretzke, H.G.:
Pysical Events in the Track Structure of Heavy Ions and their Relation to Alterations of Biomolecules.
Submitted to Adv. Space Res.

Paretzke, H.G.:
Parameters Characterizing Charged Particle Track Structures.
Proceed. IAEA Advis. Group Meeting "Atomic and Molecular Data for Radiotherapy", Wien, Juni 1988, im Druck.

Paretzke, H.G.:
Physical Aspects of Radiation Quality.
Proc. 14th Gray Conference "Low Dose Radiation - biological bases of risk assessment, Sept. 1988, Oxford, Francis + Taylor, im Druck

Paretzke, H.G., Turner, J.E., Hamm, R.N., Wright, H.A.:
Spatial Distributions of Inelastic Events Produced by Electrons in Gaseous and Liquid Water.
Submitted to Radiat. Res.

Long, K.A. Paretzke, H.G., Müller-Plathe, F., Diercksen, G.H.F.:
Calculation of Double Differential Cross Sections for the Interaction of Electrons with a Water Molecule, Clusters of Water Molecules and Liquid Water.
Submitted to J. Chem. Phys.

Paretzke, H.G.:
Simulation von Elektronenspuren im Energiebereich 0,01 - 10 keV in Wasserdampf.
GSF-Bericht 24/88, Neuherberg, 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-A-013-D

Julius-Maximilians-Universität
Würzburg
Institut für Med. Strahlenkunde
Sanderring 2
D-8700 Würzburg

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. A.M. Kellerer
Institut für Med.Strahlenkunde
Universität Würzburg
Versbacher Str. 5
D-8700 Würzburg

Telephone number: 931-201.38.36

Title of the research contract:

Development of the twin detector method and of microdosimetric concepts and methods for radiation protection.

List of projects:

1. Implementation of the twin detector method and computational studies.
2. Mathematical and numerical studies in microdosimetry.
3. Microdosimetry of heavy ion beams at the UNILAC.

Title of the project no.: 1

Implementation of the Twin Detector Method and Computational Studies

Head(s) of project:

Prof.Dr.Albrecht M.Kellerer

Scientific staff:

Dr.J.Breckow, Dr.H.Roos, J.Chen, A.Philipp, H.Friede

I. Objectives of the project:

The variance-covariance method (Kellerer and Rossi, Radiat. Res.97, 237, 1984) permits the determination of microdosimetric parameters in radiation fields of fluctuating intensity. A pair of detectors (the twin-detector) is exposed to the same radiation field and registers the energy imparted within each of the detectors simultaneously. The difference between the variance (due to the fluctuations of energy imparted) and the covariance (due to the dose-rate fluctuations of the radiation field) determines the intrinsic microdosimetric fluctuations depending only on radiation quality, i.e. the inherent properties of the radiation.

II. Objectives for the reporting period:

A prototype experimental apparatus had been designed and utilized to obtain the results which were reported after the preceding period. The twin-detector was, at this stage of the investigations, not yet of tissue equivalent material, the electronic equipment was limited in sensitivity, and the signal processing system was comparatively slow; the equipment was, therefore, not fully adequate for routine work.

In this reporting period these shortcoming were, at least partly, resolved, and first steps were carried out to establish an improved set-up which can be utilized in a broad range of practical applications. A second main aspect of the work was the comparison of the measurements with computations based on transport equations and Monte-Carlo simulations. Computations were performed for different detector geometries and sensitive volumes at various photon energies and under different conditions corresponding to changing experimental parameters.

III. Progress achieved:

A pair of new tissue equivalent cylindrical proportional counters for the twin-detector was constructed together with suitable electronic signal-processing instrumentation. For calibration of the new counters and the signal-processing the energy spectrum and the LET spectrum were determined for a collimated α-ray source (Am-241) integrated in the detector shell. On the basis of the data, the gas multiplication factors for different gas pressures and electrode voltages were evaluated.

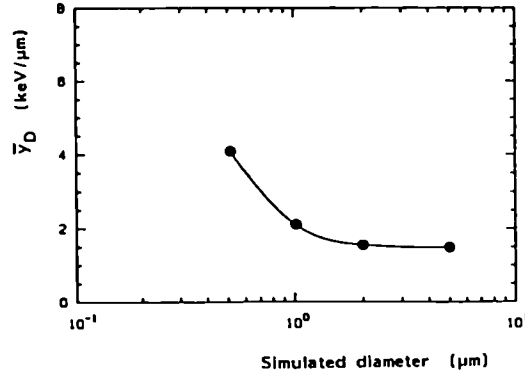


Fig. 1

Comparative measurements in a test radiation field (Cs-137, activity 185 MBq, 750 nGy/s) demonstrated substantial agreement with the results which had been obtained with the former version of the twin-detector in the same radiation field . The microdosimetric parameter, y_D, was determined in the radiation field for various gas pressures, i.e., for different simulated diameters of the sensitive volume, ranging from 0.5 μm to 5 μm (see Fig.1).

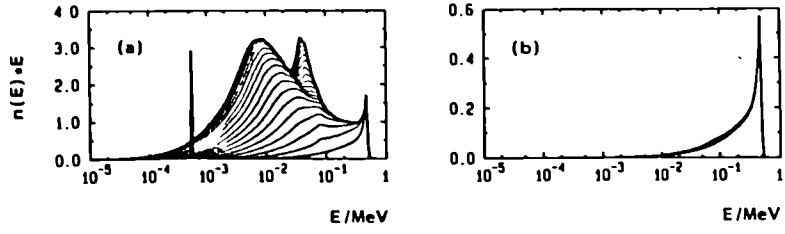


Fig. 2

The computations employed a Monte-Carlo transport code for photons in water. Volumes of different size and shape and different radiation fields were simulated. Fig.2 shows the distribution of initial energies of all generations of electrons produced by the 0.66 MeV photons of Cs-137 in an infinite volume (a), and indicates the very substantial difference to the case of a unidirectional photon beam incident parallel to the detector axis and producing only 'frist collision' electrons (b). The simulation programme is utilized to treat the actual, more complicated situation which lies in-between the two extremes.

IV. Objectives for the next reporting period:

The final version of the twin-detector device will be independent of a stationary power supply and a gas flow system. A portable detector system for on-line operation will thus be provided.

Measurements will be performed in time-varying radiation fields, e.g. pulsed accelerator beams. The simulated diameter will be variable; an effort will be made to obtain data down to about 100 nm.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Radiation Research Laboratories of Columbia University, New York, (Drs. H.H.Rossi, P. Kliauga and M. Zaider).

Radiophysical Laboratory, Aarhus Kommunehospital, (Dr. K. A. Jessen)

National Institute Radiation Protection, Stockholm (Dr. L. Lindborg)

VI. Publications:

Breckow, J., Wenning, A., Roos, H., Kellerer, A.M., The Variance-Covariance Method: Microdosimetry in Time-Varying Low Dose-Rate Radiation Fields. Radiat Environ Biophys 27, 247-259, 1988.

Philipp A., Monte-Carlo Simulations of Photon Tracks in Water. Internal Report IMSK 122/89.

Title of the project no.: 2

Mathematical Numerical Studies in Microdosimetry

Head(s) of project:

Prof.Dr.Albrecht M.Kellerer

Scientific staff:

Dr.J.Breckow, Dr.H.Roos, J.Chen, A.Philipp, H.Friede

I. Objectives of the project:

The development of microdosimetry in recent years has been greatly influenced by the new methods of the simulation of charged particle tracks. This has led to various extensions of microdosimetric concepts. However, there is still insufficient connection to work performed in stochastic geometry and stereology. To introduce mathematical methods developed in other fields into microdosimetry is a main objective of the program. Application of microdosimetric concepts to radiobiology is a second objective.

II. Objectives for the reporting period:

In the preceding reporting period a general relation for the variance of the intercept of two randomly superimposed objects has been developed. This is the mathematical theorem containing the fundamental relation in microdosimetry which expresses the dose mean event sizes of energy imparted, specific energy or lineal energy in terms of the proximity functions of charged particle tracks and the reference volume. One of the main objectives for the present reporting period was the extension of the theory, partly with direct connections to microdosimetric problems, but largely also in general mathematical directions. Two important issues continued to be the problem of systematic sampling and the relation between spatial distributions and the proximity function.

III. Progress achieved:

The proximity function and related concepts correspond in microdosimetry to the analysis of spatial patterns of energy deposition; these quantities are, therefore, closely related to simulation studies of charged particles. The earlier concepts of energy imparted, specific energy or lineal energy in finite volumes are more closely linked to experimental procedures and they appear complimentary to the use of exact spatial patterns. The fundamental relation in terms of the proximity functions has established a linkage between the two diverse aspects, and this has motivated a unified formulation of microdosimetry.

The new formulation extends the notion of specific energy to include point functions representing the inchoate distribution tion in the sense of the distribution theory. This is more than a formal extension. Largely in analogy to concepts de-veloped by Matheron, it permits the extensive use of Fourier transforms and of the so-called covariograms, to establish numerous linkages between inchoate distributions and the variables that correspond to the conventional concepts of microdosimetry.

The new method of analysis has been employed in an application to systematic sampling which has led to exact solution of the familiar problem of 'Zitterbewegung' of the variance in systematic sampling; these studies have been continued. A further application has been the problem of the reversion of the proximity function to the inchoate distribution. The product of the Fourier transform of the inchoate specific energy with its conjugate is the Fourier transform of a function closely related to the proximity function and tentatively called the vectorial proximity function. With this generalized function (spatial autocorrelation function) the reconstruction problem can be solved. In the one-dimensional case the vectorial proximity function equals the conventional proximity function; it follows that the one-dimensional reconstruction problem can be solved. In the case of higher dimensions the proximity function is obtained from the vectorial proximity function by an averaging procedure which, in the terminology of Matheron, is termed radial grading. The loss of information due to the grading corresponds to the disappearance of phase information in the Fourier space. Because of this missing information, the reconstruction problem can be solved only under certain constraints.

The generalized formulation of microdosimetry leads to more basic definitions of the fundamental quantities. Within the same context we have worked towards a unification of microdosimetric and dosimetric quantities. The aim of the studies has been the development of a concept comprising kerma, absorbed dose, and a series of intermediate dosimetric quantities. To this purpose the new quantities 'cema' and 'reduced cema' have been defined. Their definition necessitates a modification of the established concept of restricted LET which has tentatively be termed reduced LET. It is proposed to abandon the former quantity.

IV. Objectives for the next reporting period:

The work on a generalized formulation of microdosimetry will be continued, and an effort will be made to reformulate in more coherent form the definitions of basic dosimetric and microdosimetric quantities. The applications to systematic sampling will be continued.

In the dosimetric and microdosimetric applications the emphasis will be on the computation of electron fluence distributions in the CSDA, to determine characteristics of the newly introduced concepts of cema and reduced cema in relation to those of absorbed dose. This involves a general examination of the problem of electronic transient equilibrium and non-equilibrium.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Columbia University, New York, (Drs.H.H.Rossi, Dr.M.Zaider)

Gesellschaft für Strahlen- und Umwelforschung, (GSF, Neuherberg), Institut für Strahlenschutz

VI. Publications:

Kellerer, A.M.
On the Variance of Volume Estimators from Systematic Planar Sections. J.of Microscopy, to appear March 1989, Second Special Edition on Stochastic Geometry.

H.H.Rossi, Kellerer, A.M.
Intermediate Dosimetric Quantities, submitted for publication.

Title of the project no.: 3

Microdosimetry of Heavy Ion Beams at the UNILAC

Head(s) of project:

Prof.Dr.Albrecht M.Kellerer

Scientific staff:

Dipl.Phys.M.Laßmann, Dr.H.Roos, Dr.J.Breckow

I. Objectives of the project:

The project is aimed at microdosimetric studies with heavy ion beams of the linear accelerator UNILAC of the GSI (Gesellschaft für Schwerionenforschung, Darmstadt). While a variety of radiobiological projects are being performed with the heavy ion beams, there has been a lack of microdosimetric studies on heavy ions. Such studies are required to supplement and to verify computational results obtained from simulated particle tracks.

II. Objectives for the reporting period:

Radial energy distributions were determined for protons of energy up to 11 MeV. The main objective for this reporting period was the critical evaluation of the results in a comparison with results obtained by other experimental methods and a comparison with results computed at our laboratory.

III. Progress achieved:

The experimentally determined radial distributions of energy around the tracks of energetic protons were compared to earlier results obtained for lower proton energies by Ibach (GSF). As shown in the upper panel of Fig.1 a coherent systematic dependence on proton energy was found in this comparison.

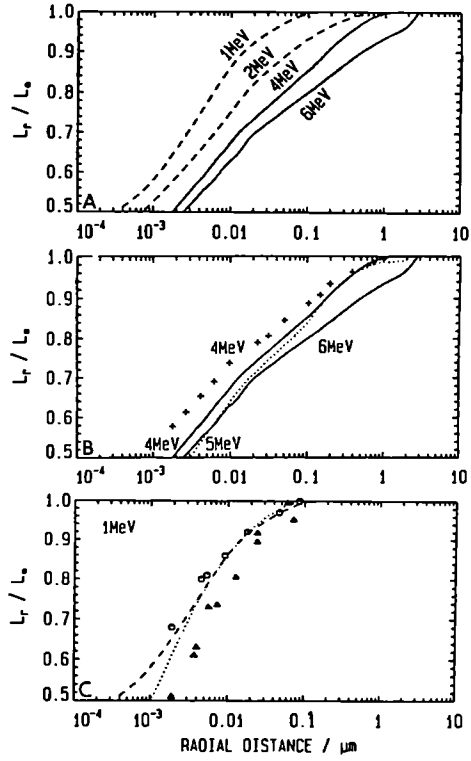


Fig. 1

In a comparison with data obtained by different experimental methods we found that results by Mills et al. (+ in the intermediate panel) correspond to considerably more narrow radial distributions, while earlier data by Wingate and Baum (/ in the lower panel) are broader than the distributions determined in our study. A tentative explanation is that the results of Wingate and Baum were subject to diffusion which led to broadening. The more narrow distributions obtained by Mills et al. near the track core appear to be due to the fact that the measurement of ioniziations corresponds to

higher effective energy cut-off values for the electron fluence. Our measurements of light emission are representative of total fluence down to about 10 eV in the measurements of light emission due to the first negative system and of electron fluence at low energies of only a few eV in the second positive system. They correspond to low energy cut-off's, i.e. they reflect energy transport even by low energy electrons, and this must lead to somewhat broader energy distributions, as they are relevant also for absorbed dose.

The comparison with computations is in line with these preliminary conclusions; some of the computed data are exemplified by the dotted lines in Fig.1. The computations are based on a simulation algorithm largely equivalent to the program of Zaider and Brenner, but extended to higher electron energies. The algorithm has been implemented for utilization on microcomputers to permit more extensive applications. The computational studies will need to be continued for a more systematic assessment of the cut-off problem.

IV. Objectives for the next reporting period:

The main objective of the work in the next reporting period is the systematic comparison of results computed for different cut-off energies. These problems are closely linked to some aspects of the work in project 2; joint algorithms and related concepts will, therefore, be utilized. The studies will also deal with the general problem of relating microdosimetric data obtained with ionization measurements to actual distributions of specific energy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Gesellschaft für Strahlen- und Umweltforschung, (GSF, Neuherberg), Institut für Strahlenschutz,

Physikalisches Institut der Universität Würzburg,

Gesellschaft für Schwerionenforschung (GSI), Darmstadt.

VI. Publications:

Laßmann, M.
Messung der räumlichen Lumineszenzverteilung zweier optischer Übergänge des molekularen Sticksdtoffes um die Bahn geladener Teilchen. Thesis, University Würzburg, 1989.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-A-008-NL

Rijksinstituut voor Volksgezondheid
en Milieuhygiëne (RIVM)
P.O.Box 1
NL-3720 BA Bilthoven

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H.P. Leenhouts
Laboratory for Radiation Res.
RIVM
P.O.Box 1
NL-3720 BA Bilthoven

Telephone number: 030-743016

Title of the research contract:

Comparative risk assessment of radiation and other mutagenic agents. Low dose relative risk of different ionizing radiations and comparison with UV radiation.

List of projects:

1. Comparative risk assessment of radiation and other mutagenic agents. Low dose relative risk of different ionizing radiations and comparison with UV radiation.

Title of the project no.:

Comparative Risk Assessment of Radiation and Other Mutagenic Agents.

II. Low Dose Relative Risk of Different Ionizing Radiations and Comparison with UV Radiation.

Head(s) of project:

H.P.Leenhouts

Scientific staff:

E.Wijngaard

M. Pruppers

K.H. Chadwick

I. Objectives of the project:

The aim of the project is to develop a comprehensive conceptual approach to permit a comparative risk assessment of the cellular effectiveness of different radiations and other mutagenic agents on the basis of the mechanisms of action. To that purpose, calculations of the radiation quality dependence of the linear term of the dose relationship will be made, and both experimentally and theoretically the similarities and differences between ionizing radiation and UV will be investigated in a consideration of the quadratic term of the dose relationship.

II. Objectives for the reporting period:

- Analysis of cell survival and transformation data after exposure to high energy ions (100-1000 Mev/μ, Z-6 to 26), using track structure model.
- Comparison of data developed in stationary CHO cells studying the effects of fractionation and delayed plating with predictions developed from the molecular hypothesis of radiation biology.
- Initiation of experiments to study the interaction of x-ray and UV induced lesions in cell survival of stationary CHO cells.

III. Progress achieved:

Track structure model:

The track structure model has been reassessed to investigate the nature of normalization factors used to adjust the theoretical formulae to experimental stopping power data and some further differences between the predictions of the model on, e.g. degradation spectrum, and other calculations published in the literature. The primary equations have been analysed and the single collision spectrum for liquid water has been renormalized and tested against several boundary conditions for consistency. The stopping power for electrons and protons has been calculated and fitted to experimental data by adjusting a single unknown factor in the equation for the maximum impact parameter. No normalization of the model to the data is now required. A modification of the equation for the calculation of the degradation spectrum has indicated that at electron energies above 200 eV a good agreement between the model calculations and the published data is achieved. At energies below 200 eV, further modifications to the stopping power are indicated. These modifications are justifiable on physical grounds and should lead to a realistic degradation spectrum over the whole energy spectrum.

Cellular response to gamma-rays and UV

Using the stationary CHO cells, experiments were initiated to investigate the interaction of gamma-rays and UV radiation. Pre-doses of UV were followed by a series of gamma-ray doses and vice versa. An interaction between the effects of the two types of radiation was found assessed by the fact that the combined effect was always greater than the additive effect of the two radiations given independently. The analysis of the results indicated that a pre-dose of UV increased the linear coefficient of the linear-quadratic gamma-ray dose response and that in both cases the interaction effect was proportional to the product of the gamma-ray dose and UV exposure. These results are in accordance with the hypothesis (Chadwick and Leenhouts, 1981. The Molecular Theory of Radiation Biology, Springer Verlag, Heidelberg.) which is based on the assumption that single stranded DNA lesions induced by each radiation type can interact with each other to produce double stranded DNA lesions which are potentially lethal.

An analysis of fractionation.

A possibility to test the behaviour of the quadratic term of the dose-effect relationship for gamma rays is an analysis of fractionated irradiation.

If cell survival(S) is a linear-quadratic function of dose (D), i.e.,

$$-\ln S = \alpha D_{tot} + \beta D_{tot}^2 \qquad (1)$$

where α is the probability that a lethal lesion is induced in the passage of a single ionizing particle and β is the probability that two independently produced sub-lesions combine to produce a lethal lesion and D_{tot} is the total dose (we assume sub-lesions are DNA single strand breaks and lethal lesions are DNA double strand breaks), then, when a total dose is given in n equal fractions ($D_{tot} = nD_f$) with time between fractions to allow a full repair of sub-lesions, cell survival (S_f) after a series of n fractions of dose (D_f) is given by:

$$-\ln S_f = n\,(\alpha D_f + \beta D_f^2). \qquad (2)$$

Equations (1) and (2) can be combined to eliminate α such that

$$\frac{1}{n(n-1)} \ln \left[\frac{S_f}{S}\right] = \beta D_f^2 . \qquad (3)$$

This equation predicts a single straight line relationship with slope β independent of the number or size of the dose fractions. Experiments have been done with doses given as single, two or three fractions with 3 hours between fractions and 24 hours post-irradiation delay before plating. The results are shown in figure 1. The results show that the experimental data are consistent with a linear-quadratic dose effect relationship with the quadratic term arising from the combination of two sub-lesions which can repair in the time between the fractions. The slight curvature in the plot might indicate that the 3-hour interval is not sufficient for complete repair of sub-lethal damage. Further experiments will be done.

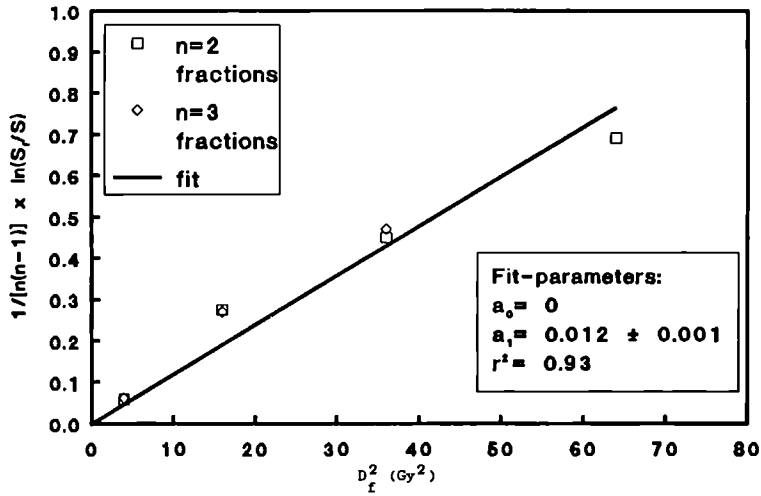


Fig. 1. Data for 2 and 3 fractions plotted according to equation 3.

IV. Objectives for the next reporting period.

- Further reassessment of the track structure model and its use to describe the radiation quality dependence of 'single-hit' detectors and DNA double strand breaks in comparison with the old model.
- When the reassessed track structure model is fully operational, the analysis of survival and cell transformation data intended for 1988 will be attempted.
- Continuation of the investigation of the interaction of x-ray and UV induced lesions in cell survival of stationary CHO cells.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Drs. J.H. Nelson and L.A. Braby. Pacific Northwest Laboratory. Richland. USA.
- Drs. A. Cebulska-Wasilewska and M. Waligorski. Institute for Nuclear Physics. Krakow. Poland.

VI. Publications

Chadwick, K.H., H.P. Leenhouts, E. Wijngaard and M. J. Sijsma. DNA double-strand breaks and their relation to cytotoxicity. In 'Quantitative Mathematical Models in Radiation Biology'. (ed. J. Kiefer) Springer Verlag (Heidelberg) 1988, 147-158.

id. (Abstract) Radiation Environment. Biophysics 27 (1988) 221.

Leenhouts, H.P. and K.H. Chadwick. Radiobiological Arguments for a Linear Dose-Effect Relationship of Stochastic Effects at Low Doses. Radiation Protection Practice (Proc. of 7th IRPA Congress) Volume III. (Pergamon Press, New York) (1988) 1215-1218.

Leenhouts, H.P. and K.H. Chadwick. The Molecular Basis of Stochastic and Non-Stochastic Effects. In Proc. of 26th Hanford Life Sciences Symposium. Modeling for Scaling to Man. Health Physics (In Press). 1988.

Chadwick, K.H. Dosimetry Concepts in Food Irradiation for the Gamma Irradiation of Food In Proc. of Health Impact, Identification, and Dosimetry of Irradiated Foods. (Eds. K.W. Bögl, D.F. Regulla, M.J. Suess) Publ. BGA. ISH. Heft 125, 1988, p. 400-404.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Com.Naz.per la Ricerca e per lo
Sviluppo dell'Energia Nucleare e
delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Contract no.: BI6-A-023-I

Head(s) of research team(s) [name(s) and address(es)]:

Dr. L. Lembo
ENEA - Lab. Applicazioni di
Dosimetria
Via Mazzini 2
I-40138 Bologna

Telephone number: 051-498350

Title of the research contract:

Track-structure detectors for neutron and alpha dosimetry.

List of projects:

1. Neutron dosimetry by damage track detector: advantages and limitations.
2. Applied personal dosimetry by chemical etched CR-39 dosimeters.
3. The assessment of low concentration of alpha emitting radionuclides.

Title of the project no.: 1.

1. Netron dosimetry by damage track detectors: Advantages and limitations.

Head(s) of project: L. Tommasino

Scientific staff: L. Tommasino and G. Torri.

I. Objectives of the project:

The major scope of this project is to demonstrate how it is possible to obtain any desired neutron-dosemeter response through a proper choice of the parameters of the electrochemical etching of damage track detectors.

II. Objectives for the reporting period:

Further investigations on the two-steps etching formed respectively of chemical and purely electrical processes.

III. Progress achieved:

1. Methodology.

The most attractive characteristic of the electrochemical etching is the possibility to choose several combinations of the etching parameters to obtain a large variety of different responses to neutrons. In particular in this project the response of the electrochemically etched CR-39 detectors has been studied using a two-steps etching formed respectively of chemical etching and purely electrical phenomena.

2. Results.

Under the two-steps etching mentioned above, track spots with uniform sizes and negligible overlapping are obtained thus providing excellent characteristics for automated spot counting. Because of these attractive characteristics systematic investigations of the spot size distributions have been made with CR-39 foils irradiated to neutrons with different energies. These distributions present a simple peak shape for all the neutron energies with the exception that of 144 keV. A pronounced tail at the low size range has appeared in all the cases. The average value of the track spot size does not increase appreciably for the neutron energy of 1.2 MeV up to 14.7 MeV. For neutron energies of 144 and 250 keV respectively the track sizes are relatively small when compared with those at high energies.

3. Discussions.

The differences in the size of track-spots for the low-energy-proton-recoils can be due to the fact that these tracks are very shallow, which characteristics seem to affect the extent of the tree formation and propagation.
Further investigations on these phenomena are important specially for a better understanding of the mechanisms of the electrochemical etching processes.

IV. Objectives for the next reporting period:

Investigations of the electrochemical etching parameters for the optimation of damage track detector response to neutrons will be continued. Particular efforts will be made to extent these investigations to detectors other than CR-39 such as cellulose nitrate and polycarbonate detectors.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

A. Castellano. University of Lecce-Italy
A. Cecchi. National Institute of Nuclear Physics-Florence. Italy.

VI. Publications:

D. Azimi-Garakani, L. Tommasino and G. Torri (1988) Further investigations on electrochemically etched CR-39 neutron detectors. Proceedings of the 14th Int. Conf. on SSNTD, 3-6 April, Lahore - Pakistan.

Title of the project no.:

Project N. 2 - Applied Personal Dosimetry by Chemically Etched CR-39 Dosimeters.

Head(s) of project:

L. Lembo

Scientific staff:

L.Lembo, O. Civolani , M. Beozzo

I. Objectives of the project:

Development of a CR-39 Neutron Personal Dosimeter for Large Dosimetry Services.

II. Objectives for the reporting period:

Full replacement of nuclear emulsions with track detectors in the Enea-Bologna Neutron Personnel Dosimetry Service.
Comparative study of background characteristics of CR-39 polymers manufactured by different factories.
Preliminary investigation on the advantages to etch in differently ways more than one area in each track detector to reduce the background inconvenients and to obtain a rough energy information of the neutron fields.

III. Progress achieved:

At the beginning of the year, in our neutron personnel Dosimetry Service, the nuclear emulsion technique has been fully replaced with the one based on CR-39 track etch detector. As previously reported,the CR-39 detectors are on one side electro-chemically etched, by means of a particular lucite cell suitable for simultaneously etching 24 CR-39 detectors 30x29 mm^2 and 600 μm thick. In routine activities, 4 cell are simultaneously used ; these are placed inside an oven kept at a temperature of 60 °C . The whole electrochemical etching treatment is automatically performed, using a three step cycle in a 6N KOH solution (8 h at 35 kV/cm and 60 Hz followed by 23 m at 35 kV/cm and 2 kHz and finally 15 m without electric field).

At present 1000 personnel neutron dosimeters are supplied by our Dosimetry Service with an issue period of 45 days.

In spite of the investigations previously developed at laboratory conditions, the management of the large number of detectors used in the operative condition has put in evidence some serious problems arising from the very high variations of the background track density and the need for improving the technique to find out better etching conditions and/or new CR-39 polymers of higher quality. To this aim , a systematic investigation has been made to evaluate the background response of different CR-39 polymers , manufactured by different factories and stored on different laboratory conditions. This activity has also been a part of a large survey of background in CR-39 detectors organized by the Eurados-Cendos at European level.

Three different CR-39 polymers have been investigated : one from Pershore Moulding (PM) - UK , one from American Acrylic (AA) - USA and the last one, to be introduced in the market in the near future , from Intercast (IC)- Italy. These three kinds of CR-39 materials have been kept in different storage conditions, so as to see the influence of storage conditions on the track background. The AA detectors were stored in a refrigerator at 4 °C for 4 months and then placed in a box with nitrogen atmosphere at 20°C for 7 months , the PM detectors were stored in nitrogen atmosphere at 20 °C for 6 months and the IC detectors were stored in nitrogen atmosphere at 20 °C for 3 months. To have a lower and more reproducible background , the electric field used in the etching cycles has been reduced from 35 kV/cm to 25 kV/cm. Eight detectors exposed to Cf-252 (4 front and 4 back sides) and 16 unirradiated detectors (8 front and 8 back sides)were simultaneously etched , so to study in the same conditions the sensitivities and background characteristics on both sides of the polymer. Figures 1,2 and 3 show the results obtained from these investigations. The IC material shows higher sensitivity on both sides compared to AA and PM , but its average background is higher and with larger variations than the other polymers. From these results it has also been observed that AA detectors show better behaviour on front and back sides

than the other too materials.

A particular micro-cell suitable for etching two areas on the same detector has been designed and constructed. This new device should allow to electrochemically etch two areas of the same detector in different conditions : in particular a low electric field for a long time will be used for one area , to detect recoils from fast neutrons , while an higher electric field for short etching time wil be used for the other area, to detect recoils from low energy neutrons.

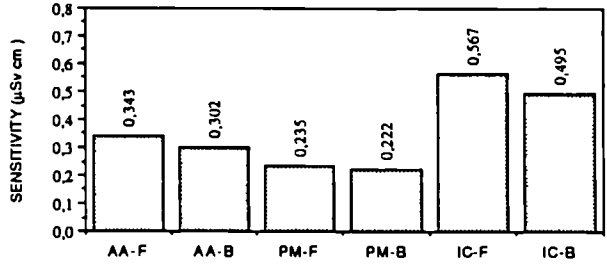


Fig 1. Cf-252 sensitivity of tested Cr-39 polymers

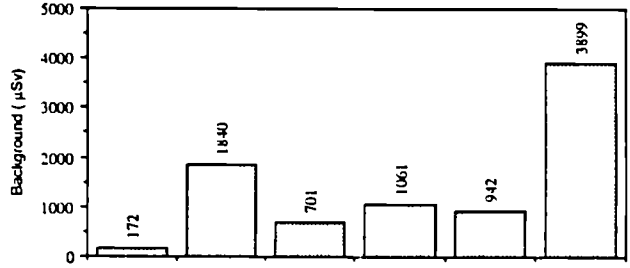


Fig 2 Average Background of different CR-39 polymers

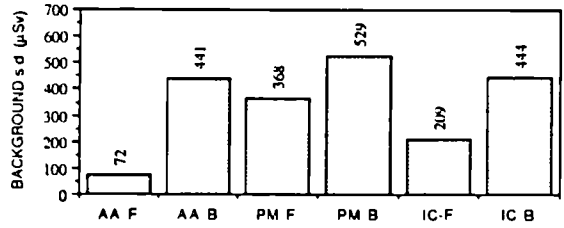


Fig 3 Background standard deviation of tested CR 39 polymers

IV. Objectives for the next reporting period:

Determination of the energy response of CR-39 neutron detectors etched with an electric field of about 25 kV/cm as presently used in the Enea dosimetry service.

Investigation on the advantages of using the designed micro-cells to overcome the background inconvenients and to have a qualitative information on the neutron energy spectra.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

NRPB, Harwell (UK)
PINSTECH - SSNTD Laboratory , Pakistan.

VI. Publications:

1) . L.Lembo " Personal Neutron Dosimetry Using Track Detectors " Lahore (Pakistan) 14th Inter. Conference on SSNTD -1988 (to be published)

2) . L.Lembo. M.Beozzo, S.Manzoor, H.A.Khan & A.Waheed
" Results from ENEA-Dosimetry Laboratory, Bologna "
Eurados-Cendos Neutron Dosimetry Background Survey -1988
(To be published)

Title of the project no.: 3

The assessment of low concentrations of alpha emitting radionuclides.

Head(s) of project: L. Tommasino

Scientific staff: L. Tommasino and G. Torri

I. Objectives of the project:

Development and applications of new detectors based on track-structure properties for the assessment of low concentrations of alpha emitting radionuclides.

II. Objectives for the reporting period:

Damage track detectors are considered not sufficiently sensitive for short-term (one week) measurements of radon exposures. The major scope of this project was to stress the simplicity of measuring short term radon exposures by spark counting larg detector areas.

III. Progress achieved:

1. Methodology.

The damage track detectors are considered not sufficiently sensitive for short-term radon measurements mainly because the detector area counted under the microscope is typically less than 20 mm^2 . Assuming valid the Poisson statistics, if the detector area is increased by a factor of one hundred (typically from 10mm^2 to 10 cm^2) the Lower Limit of Detection LLD decreases by a factor of 10. Since detector areas up to hundreds of cm^2 can be easily counted by the spark replica counter, short term radon exposures can be achieved simply by spark counting large foils of cellulose nitrate.

2. Results.

A new radon monitoring has been recently developed using a large LR-115 (9cmx12cm) enclosed in one envelope. This envelope-type radon sampler has been calibrated and used for weekly measurements in one test house.
With the results obtained after one year of weekly measurements, the advantageous characteristics of this radon monitor have been fully proven. These characteristics can be listed as in the following:

- Possibility of scanning large detector areas in a fraction of a second in a very reliable way, achieving very low detection limits.
- Possibility to turn on and off the detector simply by opening and closing an envelope.
- High accuracy for short term measurements.

3. Discussions.

For short term measurements, damage track detectors are considered not sufficiently sensitive and the diffusion barrier charcoal adsorption collector, DBCA, is used in spite of its response highly dependent on temperature and humidity.
With this project it has been extensively proven how simple it is to measure short term radon exposures by using bare LR-115 detectors and the spark counter.

IV. Objectives for the next reporting period:

Continuation of short term radon measurements both in indoor and outdoor environments. Development of new techniques for the measuremnts of low-concentrations of both man-made and alpha emitting radionuclides.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. O.C. Oppon - Ghana Atomic Commission - Ghana.
2. D. Azimi Garakani - Teheran University. Iran

VI. Publications:

O.C. Oppon, D. Azimi-Garakani, L. Tommasino, G. Torri, and S. Aziz (1988). Radon monitoring for short term exposures in indoor air. In the Proceedings of the 14th Int. Conf. on SSNTD. 3-6 April, Lahore - Pakistan.

L. Tommasino (1988). Assessment of natural and man-made alpha emitting radionuclides. Ibidem.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Contract no.: BI6-A-015-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. T.O. Marshall
Instrumentation Department
NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Telephone number: 235-83.16.00

Title of the research contract:

The implementation of new ICRU operational quantities for use in radiation protection.

List of projects:

1. Investigation into the choice of phantom shape for the calibration of personal dosemeters in terms of the new operational quantities.
2. Field measurements of photon energy and angle spectral distribution, the determination of area dose equivalent, individual dose equivalent and effective dose equivalent and their comparisons.

Title of the project no.:

Investigation into the choice of phantom shape and material for the calibration of personal dosemeters in terms of the new ICRU operational quantities.

Bead(s) of project:

Dr D T Bartlett
Dosemeter & Detector Development Group
National Radiological Protection Board

Scientific Staff:

Dr D T Bartlett
Dr P J Dimbylow
Mr T M Francis
Mr T O Marshall

i. Objectives of the project:

The new ICRU secondary quantities for external radiation exposure require the choice of suitable phantoms on which to calibrate personal dosemeters when determining their energy and angle dependence of response. ICRU have indicated that the ICRU tissue equivalent sphere is a suitable phantom for dosemeters designed to be worn on the trunk. Material of ICRU soft tissue composition cannot be produced. Also a sphere is an inconvenient shape for routine calibration. Accordingly, investigations are being carried out of the suitability of alternative materials and shapes.

ii. Objectives for the reporting period:

The objectives for the reporting period were to carry out validation experiments; to calculate backscatter data for a poly methyl

methacrylate sphere of 30 cm diameter; to prepare the final report on the investigations.

iii. Progress achieved:

1. Methodology

1.1 The International Commission on Radiation Units and Measurements (ICRU) introduced, in ICRU Report 39[1], two quantities for individual (personal) monitoring, namely individual dose equivalent, penetrating, $H_p(10)$, and individual dose equivalent, superficial, $H_s(0.07)$. These quantities are defined as the dose equivalents in soft tissue below a specified point on the body (normally taken to be defined by the position of a personal dosemeter) at depths of 10 and 0.07 mm respectively. $H_s(0.07)$ is considered to provide an adequate estimate of skin dose equivalent in the vicinity of the dosemeter. For a dosemeter worn on the anterior portion of the truck (presuming this to be representative of the most highly exposed portion of the trunk) ICRU 39 states that $H_p(10)$ can be related to effective dose equivalent, H_E, received by the trunk for radiation incident from anteriorly to laterally[1].

1.2 The calibration of a personal dosemeter/dosimetry system is the determination of the relationship of the magnitude of response of the dosemeter/dosimetry system to the magnitude of a stated quantity (for a definition of calibration, see, for example, reference 2).

$$M = RQ$$

The response function R (the inverse of the calibration factor) which relates measured value, M, to quantity, Q, is generally a function $R(E,\alpha)$ of radiation energy, E, and direction, α. It may be determined for a range of radiation energies and directions (type-testing) or for single values (routine calibration and performance assessment). The quantity Q may be chosen from many, subject to the requirement that it can be clearly and uniquely defined for a given set of primary physical radiation field quantities and can be traceable to such quantities. The family of suitable Q will therefore include effective dose equivalent

calculated for the anthropomorphic phantom derived from the MIRD model[3], H_E', (the prime here indicating that it is the calculated value for this phantom) and the ICRU new secondary quantities[1], the directional dose equivalents at 10 mm and 0.07 mm, H'(10) and H'(0.07) respectively, as well as air or tissue kerma or absorbed dose. When calibrating dosemeters on phantoms, the calibration field is determined by the primary beam with air and room scatter contributions, and by the backscattered radiation field determined by the material and shape of the phantom. The purpose of calibrating personal dosemeters/dosimetry systems is to be able to interpret or understand their response when in use. Dosemeters calibrated in terms of a free-in-air quantity, for example absorbed dose to tissue, and for which R is independent of energy, E, and incident angle, α, will indicate this quantity when in use. Dosemeters which do not respond to backscatter, or for situations where backscatter can be neglected, and where $R(E,\alpha)$ for the incident field is known, could be calibrated, also in free air, in terms of phantom related quantities such as H_E' or H'(10), and would in use, indicate these quantities for the forward 2π solid angle. More problematical is the case where a dosemeter which responds to backscattered radiation (and transmitted radiation incident from the rear 2π solid angle) is required to measure quantities such as individual dose equivalent, penetrating, $H_p(10)$ and individual dose equivalent, superficial, $H_s(0.07)$ which are defined in the body, and which are impossible to measure directly or to calculate for an individual. This is the case which is considered here.

1.3 For a dosemeter which is required to indicate an in-body quantity, calibration must, of necessity, be carried out either on a standard phantom or in free air (with knowledge of the backscattered radiation field and/or its effect) : individuals vary, and cannot be used for calibration. Three approaches have been proposed for the calibration of personal dosemeters designed to measure the new ICRU quantities:

(i) Perform dosemeter calibrations (type-tests) with dosemeters mounted, in principle, on the ICRU sphere in terms of the calibration quantities H'(10) and H'(0.07). The irradiation conditions should be such that the fields may be considered expanded and aligned as appropriate. Internationally agreed conversion coefficients from

field quantities (fluence or air kerma) to H'(10) and H'(0.07) are to be used. In practice, phantoms other than the ICRU sphere will, of necessity, be used. Corrections must then be applied to take account of differences between the backscattered radiation field at the location of the dosemeter on the practical phantom and that which would exist at the surface of the ICRU sphere and the resultant differences in dosemeter response. Alternatively, the dosemeter response in free air may be fully characterised, and its on-sphere response calculated. (The absence of a phantom may be considered as a limiting case of a surrogate phantom). The assumption is made that dosemeters calibrated on the ICRU sphere (or its more practical surrogate) in terms of the sphere quantities H'(10) and H'(0.07) will, when worn, indicate the body quantities $H_p(10)$ and $H_s(0.07)$. This approach has been proposed and/or discussed by Williams[4], Wagner[5,6,7], Kramer and his colleagues[8,9,10], Bartlett and colleagues[11,12,13,14,15], Marshall and colleagues[16], Harder[17] and Alberts and Dietze[18,19] and would seem to be close to the intentions of ICRU 39[1].

(ii) Perform calibrations with dosemeters mounted on an anthropomorphic phantom (a slab or semi-elliptical cylinder, solid or water filled may be adequately anthropomorphic for some radiation fields) in terms of the sphere quantities H'(10) and H'(0.07) (or their unexpanded analogues[20]) for which conversion coefficients are available. All dosemeters might then, reasonably, be expected to indicate these quantities when worn. Since it is considered[21] that for the same radiation fields differences between H'(10) and H'(0.07) (in the sphere) and $H_p(10)$ and $H_s(0.07)$ (in the body) will be small, dosemeters calibrated this way might be expected to adequately indicate $H_p(10)$ and $H_s(0.07)$. This approach has been proposed and followed by Jahr and his colleagues[20]. The major objection to this approach is that a non-existent physical situation is taken as the basis for calibration. This is metrologically unsound.

(iii) Perform calibrations on a practical phantom in terms of quantities defined in that phantom. If the phantom is chosen such that it is

reasonably anthropomorphic for the radiation fields considered, for example, the IAEA water phantom[22] for photon radiation incident normally, the quantities so defined may be expected to be similar in magnitude to $H_p(10)$ and $H_s(0.07)$. The dosemeter might then be expected to indicate these quantities when worn. Alternatively, it might be expected that differences in scattering of primary radiation between the calibration phantom and the human body would produce similar fractional changes in both dosemeter indication and quantity for the two irradiation conditions - calibration and use, and therefore little change in the ratio of indication to quantity. There are, however, no internationally agreed sets of conversion coefficients for these quantities, nor, indeed, full agreement as to the use of a particular phantom and therefore particular quantity. This approach has been proposed by Bohm and colleagues[23,24,25,26] and Wernli[27,28] and adopted by the IAEA[29] and the Federal Radiation Protection Commission of Switzerland[30].

1.4 The investigations reported here are devoted to the first approach outlined in paragraph 1.3. This approach is clearly favoured by ICRU. 'The calibration of the doemeters is done under simplified conventional conditions at the depth(d) (10 or 0.07 mm) in an appropriate phantom. For dosemeters worn on the trunk a suitable phantom is the ICRU sphere'[1]. We have interpreted this as meaning that a dosemeter/dosimetry system should be calibrated on the sphere in terms of the appropriate sphere quantity, e.g. dose equivalent at 10 mm in the sphere, H(10). Dose equivalent at 10 mm in the sphere, H(10), is not an expanded quantity, but will, under calibration conditions, be equal to the directional dose equivalent at 10 mm, H'(10). The approach has the advantage that agreed conversion coefficients for sphere quantities can be used and that no new quantities and sets of conversion coefficients need be created. It has the disadvantage that correction factors for differences between the backscatter radiation fields for the practical phantom and the ICRU sphere are dosemeter type dependent. Also it needs to be assumed, indeed needs to be established, that dosemeters calibrated on the ICRU sphere in terms of sphere quantities will indicate the body quantities when worn. The investigations address, therefore, two aspects of this approach : the calculation of the backscatter radiation fields to enable corrections to be made, and calculation and consideration of the variability of the ratio

$\frac{M}{H_i}$ where M is dosemeter indication and H_i is dose equivalent at 10 or 0.07 mm in phantom or body.

1.5 Calculations of the backscattered radiation fields of calibration phantoms have been made previously at the Physikalisch-Technische Bundesanstalt (PTB) and the Gesellschaft fur Strahlen und-Umweltforschung (GSF). Those at PTB were for a paraffin type substance M3, polymethyl methacrylate (PMMA) and water[31] (the backscatter factor being in terms of water kerma averaged over the primary photon spectra) as semi-infinite slab phantoms; for a tissue substitute, PEAR, polyethylene and PMMA as spheres or rectangular slab phantoms and ICRU 4-element tissue as a sphere or cylinder[9,10]. Those at GSF were for the ICRP elliptical water cylinder, the GSF anthropomorphic phantoms[2], ICRU sphere, PMMA 30 cm cube and a tissue equivalent material 30 cm cube[4] (the quantity, tissue kerma or other, for which the backscatter factor has been calculated is not stated).

1.6 The calculations reported here for incident photon radiation are of the energy and angle spectral distribution of the backscattered radiation field at the surface of the ICRU sphere, for a 30 cm cube of ICRU tissue, for a sphere and a cube of polymethylmethacrylate (PMMA) and for sphere and cube of MS20, an epoxy-resin tissue substitute material[32], closely tissue equivalent for photon and beta radiation[33,34]. The calculations allow a choice to be made of phantom shape and material and the calculation of correction factors. the results of the calculations have been validated by comparison, and observed close agreement, with the PTB results for ICRU tissue, by agreement of calculated values of tissue kerma on the sphere surface with calculations made elsewhere of H'(0.07), and agreement with experiment.

1.7 The experiments performed to validate the calculations were for cube phantoms of MS20 and PMMA and for normally incident photons : caesium-137 and either the 87 keV mean energy ISO low series or the 83 keV mean energy ISO narrow series of filtered bremsstrahlung radiation. The latter energies were chosen since it is in this incident photon energy region that backscatter is greatest. Small lithium tetraborate thermoluminescent

detectors were used and measured dose to this material was compared with calculated kerma.

1.8 Calculations have also been made of the dose equivalent at a depth of 10 mm in 20 and 50 cm diameter spheres of ICRU tissues, and the backscattered radiation fields for these phantoms and for a 30 cm side cube of the same material. The approximate invariance of the ratio of tissue kerma (or lithium fluoride or lithium borate kerma) at the phantom surface to dose equivalent at 10 mm in the same phantom permits the inference that dosemeters calibrated on the ICRU sphere in terms of H'(10) will, when worn, adequately indicate Hp(10).

1.9 The calculations of dose equivalent distributions in ICRU 4-element tissue spheres of diameters 20, 30 and 50 cm were made using the previously documented program DEIPHOS[35,36]. Incident photon energies were from 10 keV to 10 MeV for 30 cm diameter, 15 to 662 keV for 20 and 50 cm diameters, and for angles of incidence from 0 to π radians in increments of $\pi/16$. augmented by additional runs for $\pi/32$. This program was modified to calculate the energy and angle spectral distribution of the backscattered photon radiation field for incident photon energies of 15, 25, 50, 75, 100, 150 and 662 keV.

1.10 The spectral distributions of particle number and fluence in energy and angle were calculated for energy increments of, generally, 5 keV, and of angle cosine increments of 0.1. For cube phantoms these were calculated for a scoring disc of radius 2.5 cm centred on the 'front' surface, and for a disc 10 mm away from the surface. For sphere phantoms, calculations were similarly made for a 2.5 cm radius 'cap' on the surface and at 10 mm from it, and also for a 'cap' defined by an angle of 15° subtended at the sphere centre and for annular scoring strips defined by angle increments of 10° up to 95°. Either 10 or 20 million 'histories' were run for each phantom and incident energy. The uncertainty resulting from the Monte-Carlo statistics was generally less than 1%.

1.11 The fluence spectral distributions in energy (i.e., integrated over angle) have been interpolated to single energies of interest and folded with the calculated spectra[37] for the ISO Narrow Series of reference filtered x-radiation.

2. Results

2.1 Detailed results are given in appendices to this report. Appendix A contains photon conversion coefficient data from fluence to dosimetric quantities (air, ICRU tissue, lithium fluoride and lithium tetraborate absorbed dose) from Hubbell[38]; sphere quantities (H'(10) and H'(0.07) as functions of energy and angle taken from ICRP 51 or calculated by Dimbylow[11,35,36,40]; and for an anthropomorphic phantom (Adam and Eva) taken from ICRP 51[39]. Appendix B contains the photon spectra input data (ISO narrow series bremsstrahlung) taken from Iles[37], and folded with dosimetric quantities (air, ICRU tissue, lithium fluoride and lithium tetraborate absorbed dose) and sphere quantities (H'(10) and H'(0.07) as functions of energy and angle. Appendix C contains, for the ICRU sphere, a cube of ICRU tissue and for the calibration phantoms, the data on the backscatter radiation field. An example is given of the data format for the energy and angle spectral distributions obtained for the backscattered field. Full dosimetric data are given: fluence backscatter factors, absorbed dose, (air, ICRU tissue, lithium fluoride and lithium tetraborate) backscatter factors at phantom surface and 10 mm in front of the surface for different photon angles of incidence for monoenergetic incident photon and for the ISO narrow series. Appendix D gives data on the dependence of H'(10) and H'(0.07) and backscatter factors on phantom size. Appendix E gives the results of the experimental investigations of backscatter.

2.2 Figure 1 shows, (as a function of incident photon energy), the tissue kerma backscatter factor for the different phantoms relative to that for the ICRU sphere for the same energy and angle of incidence.

2.3 Table 1 gives ratios of surface tissue KERMA to dose equivalent at 10 mm for ICRU tissue spheres of diameters 20, 30 and 50 cm and surface and 10 mm tissue KERMA per unit incident photon fluence for these phantoms and also for a 30 cm cube of ICRU tissue and for an anthropomorphic phantom[41].

2.4 A comparison has been made between calculated and experimental values of backscatter for lithium tetraborate KERMA. Measurements were for incident ^{137}Cs radiation and for 87 keV mean energy ISO low series and 83 keV mean energy ISO narrow series bremsstrahlung for a 30 cm cube MS20 or

PMMA phantom. Calculated and experimental values showed agreement within uncertainties.

3. Discussion

3.1 Three approaches have been taken to the validation of the calculations. Firstly, comparison has been made between calculations of tissue kerma at the surface of the ICRU sphere using the backscatter field data and calculations by Dimbylow[35,36,40], by Williams[42], and by Grosswendt[43,44]: there is agreement to within 2-3%. Secondly, a close comparison was made between the results of the backscatter calculations reported here and those made at PTB[45]. There was good agreement. Thirdly, experimental validation was obtained (see 2.5 above and Appendix E). There is also general agreement with other independent studies of the influence of phantom shape[46].

3.2 Figure 1 shows how the shape (cube or sphere) and material (MS20 or PMMA) influence the backscatter field relative to the ICRU sphere. There is a clear advantage of MS20, or similar material, over PMMA as a surrogate material for ICRU tissue. The maximum difference at any incident energy or angle considered, between the MS20 sphere and the ICRU sphere was 3% for total fluence or kerma (incident plus backscattered). In many instances, corrections may be neglected. The results from other laboratories has also shown the suitability of water and the unsuitability of polyethylene[31]. The advantage of a spherical phantom is shown. Broadly, at lower photon energies differences in material are more important, at higher photon energies differences in geometry/mass are more important. The calculations allow corrections to be applied for material or geometry/mass differences. By relating calibration fields to those of the ICRU sphere, one has the advantage of reference to an internationally recognised phantom and conversion coefficients.

3.3 The backscatter factors show little difference, for a given radiation field and phantom, between the kerma quantities calculated. This gives confidence that, to a good approximation, correction factors based on these tabulated values may be used for dosemeters whose detecting elements are reasonably tissue equivalent or air equivalent. Where possible, experimental verification would be desirable.

3.4 Detailed consideration of the energy and angle spectral distributions show the close similarity of the backscattered radiation fields for an MS20 sphere and for the ICRU sphere. It may therefore be concluded that for any personal dosemeter, whether tissue equivalent or not, calibration on a 30 cm diameter sphere of MS20 will closely mimic calibration on the ICRU sphere and that response functions in terms of ICRU sphere quantities so obtained will have deviations of less than 5% from those which would be expected for the ICRU sphere.

3.5 The results of the calculations indicate that the ratio of kerma (tissue, lithium fluoride or lithium borate) at a phantom surface to dose equivalent at 10 mm in that phantom is sufficiently independent of phantom shape to give support to the thesis that a dosemeter calibrated on the ICRU sphere in terms of H'(10) will adequately indicate $H_p(10)$ when worn (Table 1). The calculations also show that both dose equivalent at 0.07 mm and dose equivalent at 10 mm are relatively insensitive to sphere diameter (ICRU tissue) over the range 20 to 50 cm and for the energy range 15 to 662 keV. The maximum deviations from H'(0.07) and H'(10) is 11%. A dosemeter which is relatively insensitive to backscatter and has been calibrated in terms of H'(0.07) and H'(10) will indicate these quantities, to some reasonable approximation, when worn. The indicated quantities should be taken to be $H_s(0.07)$ and $H_p(10)$, nevertheless.

3.6 For dosemeters which are sensitive to backscatter, it should be noted that backscatter fluence and kerma decrease by about a factor of two when moving from the surface to a separation of 10 mm. The change in backscatter which could be produced by different methods of attaching dosemeters or by different thickness of clothing may obscure any differences resulting from material or shape of the body compared with calibration phantom.

iv. Other research group(s) collaborating actively on this project:

Contact has been maintained and discussions held with groups at Physikalisch-Technische Bundesanstalt, Braunschweig (Heads of research teams: Prof. Dr S Wagner and Prof. Dr R Jahr).

Table 1

Effect of Phantom Size and Shape on Dose Equivalent at Surface H_{surf} and at 10 mm H(10) per Unit Fluence (pSv cm²)

Photon Energy (keV)	ICRU Tissue Spheres 20 cm HSURF	20 cm H(10)	30 cm HSURF	30 cm H(10)	50 cm HSURF	50 cm H(10)	ICRU Tissue Cube HSURF	ICRU Tissue Cube H(10)	Anthropomorphic* Phantom HSURF	Anthropomorphic* Phantom H(10)	Ratio of K_a to Dose at Depth of 10 mm in Phantom ICRU Tissue Spheres 20 cm	30 cm	50 cm	Cube
15	3.07	0.822	3.08	0.827	3.09	0.825	3.09	0.835	3.46		3.73	3.73	3.75	3.7
25	1.14	0.908	1.15	0.906	1.16	0.908	1.18	0.924			1.26	1.27	1.28	1.27
50	0.455	0.484	0.481	0.518	0.505	0.551	0.519	0.550	0.550		0.94	0.93	0.92	0.95
75	0.433	0.479	0.465	0.505	0.498	0.541	0.512	0.547			0.90	0.92	0.92	0.94
100	0.527	0.562	0.563	0.610	0.601	0.666	0.616	0.645	0.634		0.94	0.92	0.90	0.96
150	0.800	0.836	0.843	0.885	0.894	0.940	0.920	0.936	0.975		0.96	0.95	0.95	0.98
662	3.60	3.57	3.66	3.69	3.75	3.78	3.77	3.76			1.01	0.99	0.99	1.00

* Different computer program

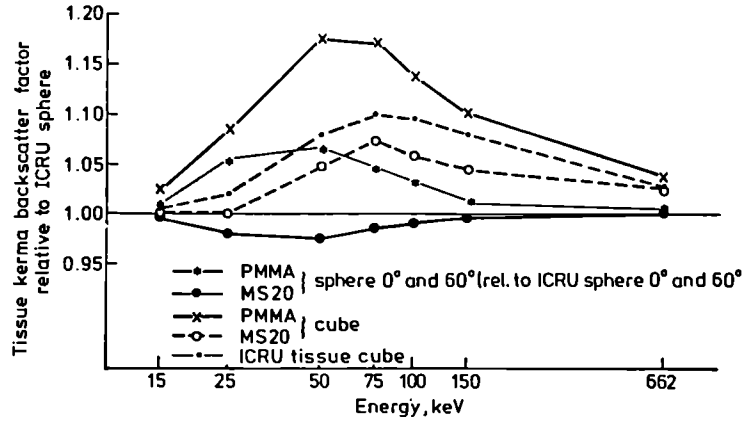


Figure 1 : Effect of Phantom Shape and Material on Backscatter

v. Publications:

Bartlett, D T, Dimbylow, P J and Francis, T M. 'Calculations of the Energy and Angle Dependence of Response of a Simplified Design of Photon Personal Dosemeter and its Relationship with the Recommended ICRU Calibration Quantity'. Radiat. Prot. Dosim. 16(4), 319-323 (1986).

Bartlett, D T, Francis, T M, and Dimbylow, P J. 'The Calibration of Personal Dosemeters'. Radiat. Prot. Dosim. (in press) (1988).

Bartlett, D T. 'The Routine Calibration of in Terms of the New ICRU Quantities of Personal Dosemeters to be Worn on the Trunk'. Radiol. Prot. Bull. (in press) (1988).

Bartlett, D T. 'Implementation of Dose Equivalent Operational Quantities in Radiation Protection Practice'. Radiol. Prot. Bull. 97, pp16-24 (1988).

Marshall, T O, Bartlett, D T and Burgess P H. 'Current and Future Instrument and Dosemeter Designs to Measure the New ICRU Radiation Quantities'. J. Soc. Radiol. Prot. 7(3), pp107-118 (1987).

Bartlett, D T, Burgess, P H, Francis, T M, Dutt, J C and Dimbylow, P J. 'The Energy and Angle Dependence of Response to Photons of the NRPB Thermoluminescence Dosemeter in Terms of the New ICRU Quantities'. Radiat. Prot. Dosim. 17, pp29-31 (1986).

Bartlett, D T. 'Interrelationships of the Proposed ICRU Operational Radiological Protection Quantities and Effective Dose Equivalent for Practical Radiation Fields'. Radiat. Prot. Dosim. 12(2), pp155-157 (1985).

Bartlett, D T and Greenhalgh, J R. 'Calculations of Effective Dose Equivalent, Ambient Dose Equivalent and Individual Dose Equivalent for a Set of Reference Neutron Spectra and Field Geometries'. 50(4), pp548-552 (1986).

Bartlett, D T. 'Dose Equivalent Conversion Coefficients, Instrument and Dosemeter Responses for a Set of Neutron Radiation Field'. Radiat. Prot. Dosim. 15(4), pp273-278 (1986).

Title of Project No.

Field measurements of photon energy and angle spectral distributions; the determination of ambient dose equivalent, individual dose equivalent penetrating and effective dose equivalent, and their comparisons.

Head of Project

Dr. D.T. Bartlett, Group Leader Dosemeter and Detector Development Group, National Radiological Protection Board.

Scientific Staff

Mr P H Burgess, Dr D T Bartlett, Mr D R McClure.

I Objectives of the project

The objective of the project is to determine the degree of approximation to effective dose equivalent which is afforded for practical radiation fields by ambient dose equivalent and by individual dose equivalent penetrating.

II Objectives for the reporting period

The objectives for the reporting period were to complete the analysis of the results of measurements made at a number of establishments and sites and to prepare a final report.

1. Methodology

1.1 For photon radiation, instrumentation has long been available which can measure radiation quantities with sufficient accuracy to meet legislative requirements, at least for the majority of workplace environments. There has, therefore, been little incentive to make energy and angular distri- bution measurements of practical photon fields. This contrasts with the situation in neutron dosimetry, where some knowledge of the spectrum is required to get the best estimate from practical neutron monitoring instruments[1].

1.2 However, there is increasing interest in energy and angular distributions, caused partly by the evolution of new monitoring quantities and partly by the general tendency to reduce dose limits. This project was designed to produce equipment and techniques which would allow simple and direct measurement, in a relatively straightforward way, of practical photon fields, and that would produce data in a form that could be used to predict effective dose equivalent for a worker as well as the values of the various new monitoring quantities[2]. In addition, it was hoped that the data would indicate that some relaxation of the metrological qualities of instruments was permissable which could lead to improvements in instrument characteristics such as robustness, reduction in weight and improvement in ease of use.

1.3 The areas investigated were chosen to be representative of the situations in which a significant proportion of worker effective dose equivalent was received. They were a radiopharmaceutical preparation laboratory (Amersham International Plc), an industrial radiography compound (David Brown Gears), a hospital radiology room (Gloucestershire Royal Hospital) and a nuclear fuel reprocessing plant (British Nuclear Fuels plc, Sellafield). It is gratifying to note that all the organisations that were approached agreed to cooperate and showed great enthusiasm for the project.

1.4 The purpose of the project was to produce energy and angular distribution information on practical radiation fields. There are many ways of producing such information. The most important criteria were that the method chosen was portable and caused the minimum disturbance to the operator of the facilities under study. The most obvious method, conventional semiconductor or sodium iodide spectrometry, was rejected for a variety of reasons, which included the bulk and inconvenience of the apparatus, the difficulty of deconvoluting relatively featureless scatter spectra, i.e. determining the actual incident radiation spectrum from the recorded signal, and the cost and fragility of the equipment. Another option considered was to use an array of passive dosemeters such as the standard film badge or some form of thermoluminescent dosemeter (TLD), but this was rejected because it would require a long integration time. During this period such a device would have to be monitored in a fixed position and would inevitably be rather bulky. It would thus be an excessive, long term nuisance

to the operator. The method chosen, which is both compact and easy to use, uses a range of filtered Geiger Muller detectors mounted in a lead collimator. This system does not offer particularly good energy resolution but, for the purpose of this project, relatively crude spectral information was acceptable, provided it could identify situations where significant divergence of the different radiation quantities occurred and where deficiencies in instruments with restricted radiological characteristics i.e. limited energy or polar responses, were likely to be significant.

1.5 The method takes advantage of the large increase in response at low photon energies of uncompensated GM detectors, i.e. detectors as manufactured. For x and γ dosimetry GM detectors are normally compensated, i.e. they are provided with filters which produce a relatively uniform response which is independent of energy over the range 50 keV to 1.25 MeV. Many instruments using such detectors are in operational use throughout the world. However, uncompensated detectors have a response which varies dramatically with energy. This is illustrated in Figure 1, which shows the response, in terms of air kerma, of the ZP1430, manufactured by Philips Components, which was one of the detectors used. The main use of this type of detector which has a thin mica end window is the detection of relatively non penetrating radiation such as beta radiation, a typical use being the measurement of beta activity on air sampler filter papers. It is possible to change the response of such a detector by covering the end window by filters of different material and thickness and with holes of different diameter. This is analogous to the production of a typical energy compensation filter but differs in the sense that the aim is not to produce a flat energy response in terms of the quantity of interest but to produce, by using different filters, a family of response curves which are substantially identical for relatively penetrating radiations ($\geq$200 keV) but which diverse considerably at lower energies. By subtracting the different count rates from each other one can also produce differential information on dose rates within relatively restricted energy bands, and this information can be used to intercompare the different monitoring quantities in the energy range where differences are most significant and where instrument characteristics have the greatest influence i.e. 10 to 200 keV.

1.6 Three standard Philips Components GM detectors were used, two of type ZP1451 and one ZP1430. These detectors were chosen because their window area is relatively large compared to their sensitive length. They were mounted in holes milled in a 51 mm x 51 mm x 152 mm lead block (Figure 2). The back of the block was covered by an additional 10 mm thick lead sheet through which the connections pass via small holes with diameters of 2.5 mm. The block was mounted on the pan and tilt head of a substantial photographic tripod. Each detector was provided with a BNC socket which was initially connected to a set of counters etc mounted in a NIM bin. Early on in the programme it was released that the NIM bin was too heavy, too difficult to

protect from contamination and required a mains electricity supply. Accordingly it was replaced by three Mini Instruments type 6.90 battery powered scaler ratemeters which were mounted in a frame and could easily be carried by one person. The final version comprised, then, a reasonably portable piece of equipment which could be carried by two people with ease and, in extremes, by one person, which was independent of mains electricity supply and which was also easy to protect from contamination.

1.7 The filter designs were produced using routines developed by NRPB for the production of energy compensated GM detectors for commercial companies. The principal of energy compensated is that different thicknesses of different atomic energy attenuation characteristics. For example, low atomic number materials, such as carbon, have attenuation characteristics which change only slowly with energy, while high atomic number materials, such as tin and lead, have attenuation characteristics which are not greatly different from those of carbon, etc at high energies, for an equivalent mass, but which increase dramatically at low energies. This is illustrated in Table 1, using data derived from Hubbell[3]. The mean value of μ_{en}/ρ and μ/ρ is used as this provides a good fit to experimental data derived from instrument design. Clearly, different materials have the same transmissions for high energy (1 MeV) radiation but vary by a factor of 10^{12} for low energy radiation (30 keV). The additional factor available in the design of filters is the opportunity to use holes through some or all of the filter elements. By balancing materials, thicknesses and hole diameters, then, it is possible to produce quite different responses from identical detectors.

1.8 This process was followed in this case. The aim was to produce three energy responses which disagreed one from the other only over quite restricted energy ranges. The results are illustrated in Figure 2. Detector 1 has a response which is relatively flat in terms of air kerma for radiation energies above 30 keV. Detector 3 has a matching response for energies less than 60 keV and greater than 180 keV. The polar responses in this energy range, i.e. the variation in count rate with angle of incidence of the radiation beam, are also similar. The air kerma component in this energy range can therefore be obtained by subtracting the count rate from detector 1 from that of detector 3 and dividing the result by the mean difference in response of the two detectors. Similarly the response of detector 2 matches that of detector 3 for energies greater than 60 keV, but for lower energies the response of detector 2 falls off much more rapidly. The air kerma rate in the 10 to 60 keV region can thus be obtained by subtracting the count rate from detector 2 from that for detector 3 and dividing by the mean difference in response. The remaining component, that greater than 180 keV, is produced by dividing the count rate for detector 1 by its mean response to obtain a notional total kerma rate and then subtracting the other 2 components calculated previously. Additional information was provided for the

dominant exposure direction by covering detector 1 with an additional 6 mm thick lead filter. This produced a measure of the very penetrating component as this thickness of lead corresponds to the half value layer for 400 keV radiation.

1.9 The subtraction routines are described in Table 3 and the resulting energy responses are shown in Figure 4. The final values of the response factors were determined using a computer simulation. The measured response factors for each detector were stored in memory and a wide variety of radiation spectra were considered. The subtraction routines were modified until the values in the 10 to 60 keV, 60 to 180 keV and greater than 180 keV bands generated by the subtraction routine agreed most accurately, on average, with the input spectra. Some typical input spectra and results are shown in Table 4. It can be seen that, in general, the results agree to within ±20%.

1.10 The values for response factor are for radiation incident parallel to the detector axis. In order to generate information on the angular distribution of the radiation field, the responses of the detectors were designed to drop to zero, or near zero for radiation incident at more than 45° to the detector axis. Some typical experimental results are shown in Table 5. At any particular orientation, the detectors viewed a solid angle corresponding to a 90° cone; this allowed the user to general 6 sets of data for any measurement position, without significant overlap at all bar the higher energies. These data were produced by pointing the detectors, in the horizontal plane, in either the dominant direction of the radiation field, or, in a building where a large number of sites was investigated, in a particular reference direction, and then taking measurements at 90°, 180° and 270° to this direction, viewed clockwise from above. Two other measurements were performed with the detectors facing virtually vertically upwards and downwards. Additional measurements were also made in cases where the radiation was incident at awkward angles, e.g. at 45° above or below the horizontal.

1.11 The facilities used in the design and development of the detectors were those operated by the Radiation Metrology Group of NRPB. All dose rates etc were measured using equipment with calibrations traceable to national primary standards and for which uncertainties are carefully documented. The radiation qualities used were: the ISO narrow series of reference filtered x radiations covering the energy range 33 keV to 248 keV; some of the ISO narrow series of reference fluorescence x radiations in the range 9.88 keV to 25 keV; ^{137}Cs and ^{60}Co γ radiations, both of which were provided by highly collimated sources. Additional testing also determined the count rate/dose rate characteristics of each detector and scaler ratemeter combination. This allowed operation in relatively intense radiation fields and also minimised errors when comparing count rates from detectors at points of maximum response difference.

1.12 The end result is a piece of equipment which can, in a relatively crude manner, determine the energy and angular distribution of practical radiation fields at dose rates corresponding to normal operational levels i.e. 2 μGy h^{-1} to a few hundred μGy h^{-1}.

1.13 The equipment and analysis described above produces data in terms of air kerma rate. It is necessary to convert this data into the quantites of interest, effective dose equivalent, H_E, ambient dose equivalent, $H^*(_{10})$, and directional dose equivalent measured at a depth of 10 mm, H'(10). These monitoring quantities represent the effective dose equivalent, the indication of survey instrument and a quantity close to that measured by a personal dosemeter worn on the body. The coefficients used were estimated using data from ICRP publication 51[4] and were calculated for the logarithmic means of the energy ranges (energy 'bin' limits). The conversion coefficients are calculated for these energies using a 4 point Lagrangian log-log interpolation routine. The directional dose equivalent H'(10) is used as a surrogate for H_p (10), the individual dose equivalent, penetrating.

1.14 The orientation of a worker was chosen to correspond to the one in which he would spend most of his time in the area. Frequently this involved facing the radiation source, but other orientations do occur. Where the worker is rarely stationary, a rotationally isotopic exposure is appropriate i.e. equal exposure from any direction in the horizontal plane. The convention used in ICRP 51 was adopted: AP is anteroposterior (front to back); PA is the opposite; LAT is lateral (from one side to the other); ROT is rotationally isotropic. One extra indication is used, FH (feet to head), for radiation incident from beneath the worker's feet, as indicated in Table 7.

1.15 The orientation information was then used in conjunction with measured energy and angular distribution data and the derived conversion coefficients, for the appropriate direction of irradiation, in Table 6 to calculate effective dose equivalent and directional dose equivalent. The value of ambient dose equivalent was also calculated by summing the various angular components in each energy band and then employing the appropriate conversion coefficients.

1.16 The calculated dose equivalents are only approximate. However, the results serve to identify situations with a significant low energy component or situations in which it is unlikely a worker will be facing the main radiation source. Such circumstances will tend to generate large differences between the various monitoring quantities and effective dose equivalent.

2. Results

2.1 Measurements were made on the premises of: British Nuclear Fuels plc, Sellafield; Amersham International plc, Amersham; David Brown Gears, Peniston, and Gloucestershire Royal Hospital. As

was discussed earlier, these organisations have representative workplace radiation fields.

2.2 The areas that were selected for study were those which were normally or frequently occupied. They were not generally the areas of highest dose rate which were accessible. Typical dose rates in the industrial plants were in the range 1 to 50 μGy h^{-1}. Those in the hospital were much higher as they involved short term fluoroscopic examinations, during which the hospital staff wear protective lead aprons etc. In most cases the radiation came from only one of the quadrants investigated and normally, again, this was the one which would be predicted from the position of sources, shielding, apertures etc. In some cases, however, radiation was incident over a wide angle and in some cases was incident in unexpected directions.

2.3 The results are given in Table 7. In BNFL, the radiation field is generated by high energy sources which are either well shielded, in the case of areas involved in reprocessing, or are relatively low activity sources, i.e. fuel manufacture. These produce a relatively high energy spectrum at worker positions with one quadrant generating at least 75% of total air kerma in 65% of cases.

2.4 At Amersham International, the majority of exposures are to high energy radiation, as demonstrated by values of H_E per unit air kerma which are less than 1.2. In some cases this holds true even when quite large quantities of low energy emitting material is handled, such as ^{125}I and ^{241}Am, because, in the case of ^{125}I, a short lived energetic nuclide, ^{126}I, is present as a contaminant, and, in the case of ^{241}Am, ^{226}Ra is stored in the same area.

2.5 In the hospital there are no significant high energies present, because the primary radiation source is a medium energy x-ray set. The main characteristic of this source is that the main direction of irradiation is backscatter from the patient, in this case represented by a 20 litre water filled phantom, and this radiation is reduced by between one and two orders of magnitude by the protective aprons.

2.6 In some ways the industrial radiography facility at David Brown Gears produces the most complicated pattern from the simplest source. The walls of the compound are extremely effective at shielding direct radiation and the main route of exposure is via the access maze, which allows once- and twice-scattered photons to reach the operator. Radiation emerging from the relatively thin removeable roof can also undergo air scatter. This leads to photon energies in the 150 keV to 250 keV region for the ^{60}Co source and the 100 keV to 200 keV region for the ^{192}Ir source. This is evident in the very much lower values of H_E compared to H^*_{10}.

3. Discussion

3.1 It is clear that worker orientation, rather than the incident spectrum, is the main influence on the ratio of H_E to the various monitoring quantities.

3.2 The majority of existing instruments, which were designed to measure air kerma rate and which are scaled in μSv h^{-1}, on the basis that 1.13 μSv = 1 μGy, can certainly be used to predict the value of H_E, normally within 25%, even for AP radiation. Recent instruments, with responses optimised for the measurement of H*(10) and calibrated using ^{137}Cs γ radiation, on the basis that 1.19 μSv = 1 μGy will tend to produce a slightly larger, but not serious, overestimate, even for AP radiation, but they have the advantage that they will predict personal dosemeter results more accurately, at least in theory.

Acknowledgements

The authors are grateful to all the organisations that provided facilities for this work, and especially to Dr A Britcher of BNFL, Mr D Noden of David Brown Gears, Mr T George of Amersham International and Mrs P M Husband at Gloucestershire Royal Hospital.

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3. Hubbell, J.H., Photon Mass Attenuation and Energy-absorption coefficients from 1 keV to 20 MeV, Int. J. Appl. Radiat. Isot. 33 1269-1290 1982.

4. International Commission on Radiation Protection. ICRP Publication 51, Data for Use in Protection Against External Radiation. (Oxford : Pergamon Press) (1987).

List of Figures

IV Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

'The Measurement of Practical Photon Radiation Fields and Calculation of Ambient and Effective Dose Equivalent'. To be published in Radiat. Prot. Dosim.

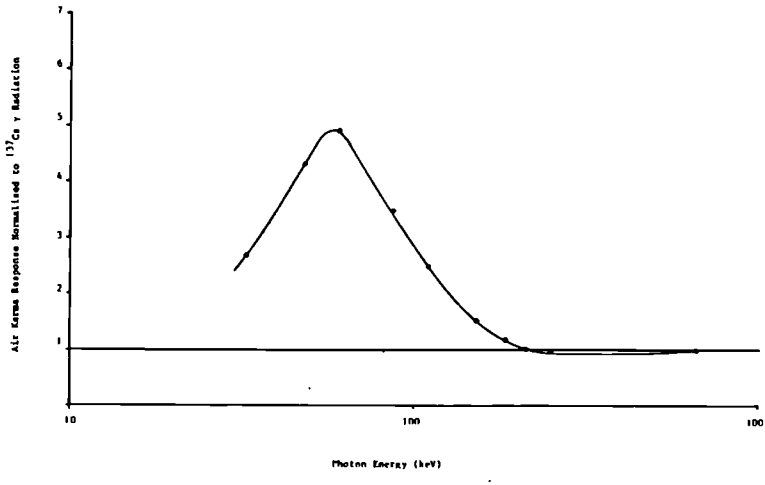

Figure 1 : Photon energy response of ZP1430 detector in terms of air kerma

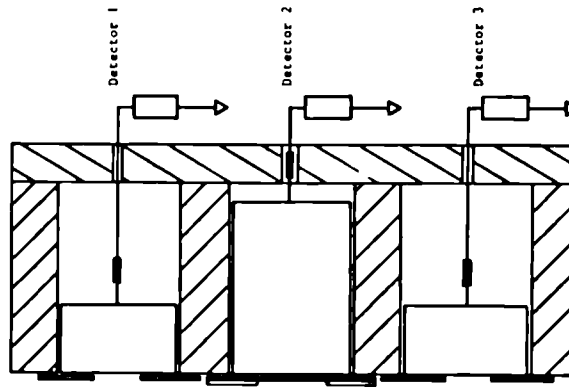

Figure 2 : The detector arrangement

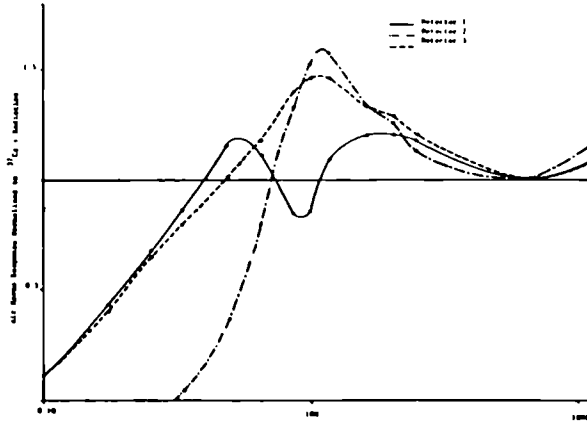

Figure 3 : Energy responses of filtered detectors in terms of air kerma

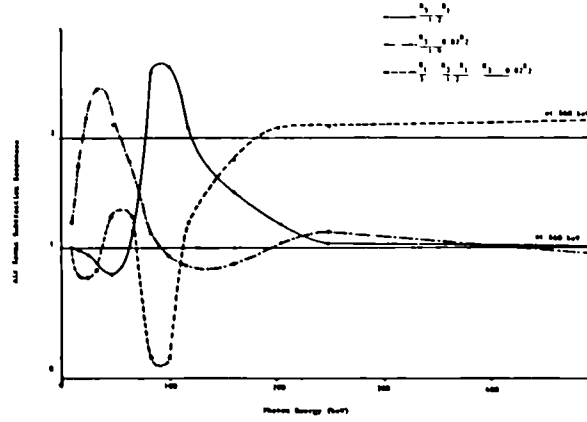

Figure 4 : Subtraction factors in terms of air kerma

Table 1 : Transmission of some typical materials at a mass cross section of 10 kg m^{-2}

Material	Transmission at indicated energy (keV)			
	30	100	300	1000
Carbon	0.88	0.94	0.95	0.96
Aluminium	0.37	0.92	0.95	0.96
Copper	4×10^{-5}	0.69	0.94	0.96
Tin	1×10^{-11}	0.24	0.89	0.96
Lead	1×10^{-12}	0.03	0.72	0.95

Table 2 : Filter compositions

Detector number	Detector type	Filter material	Thickness (mm)	Hole diameter	Hole area (% of window)
1	ZP 1451	Tin	2.16	16	33
2	ZP 1430	Copper	0.53	no hole	no hole
		Tin	0.92	23	68
		Tin	0.15	15	29
3	ZP 1451	Tin	0.15	16	33
		Copper	1.85	16	33

Table 3 : Method of subtraction and response factors related to air kerma

Energy range (keV)	Generated by*	Response factor (counts $s^{-1}/\mu Gy\ h^{-1}$)
Full	R_1	3
≥ 180	$\frac{R_1}{3} - (\frac{R_3 - R_1}{1.2}) - (\frac{R_3 - 0.82\ R_2}{1.9})$	1
60 - 180	$R_3 - R_1$	1.2
10 - 60	$R_3 - 0.82\ R_2$	1.9
Very hard	R_1 covered with extra lead	2

*R_1, R_2, R_3 are the count rates of the detectors.

Table 4 : Computer simulation of input spectra and comparison with resulting energy band values

Band centre energy (keV)	Dose input at specified band centre energy									
1250	200	200	200	200	200	0	0	0	0	20
660	-	170	170	170	170	0	0	0	0	17
248	-	20	20	20	20	20	0	0	0	0
205	-	-	10	10	10	10	0	0	0	0
161	-	-	-	10	10	10	10	0	0	0
118	-	-	-	10	10	10	10	10	10	0
100	-	-	-	10	10	10	10	10	10	0
83	-	-	-	-	10	10	10	10	10	0
65	-	-	-	-	5	5	5	5	0	0
48	-	-	-	-	5	5	5	5	0	20
33	-	-	-	-	5	5	5	0	0	20
25	-	-	-	-	5	5	5	0	0	20
17	-	-	-	-	0	5	5	0	0	0
10	-	-	-	-	0	5	5	0	0	0
Total dose	200	370	400	430	460	100	70	40	30	97
Dose 10-60 keV	0	0	0	0	15	25	25	5	0	60
60-180 keV	0	0	0	30	42	42	42	32	30	0
> 180 keV	200	390	400	400	403	33	3	3	0	37
Resulting energy band values										
10-60 keV	0	0	0	0	20	28	24	8	0	68
60-180 keV	5	15	17	46	58	45	41	38	39	0
> 180 keV	216	405	413	418	416	26	0	0	0	26

Table 5 : Polar response of detector head

Angle (Q)	Reponse normalised to Q = 0 for the indicated radiation energy (keV)								
	48			109			248		
	1	2	3	1	2	3	1	2	3
0	1	1	1	1	1	1	1	1	1
10	0.77	1.04	1.14	0.91	1.23	1.03	1.00	1.05	0.94
20	0.96	0.85	0.94	0.90	1.12	0.98	0.89	0.97	0.92
30	0.74	0.56	1.09	0.71	0.93	0.90	0.81	0.90	0.83
40	0.38	0.33	0.73	0.41	0.72	0.68	0.68	0.79	0.73
50	0.11	0.18	0.28	0.26	0.53	0.43	0.53	0.63	0.59
60	0.02	0.09	0.02	0.15	0.33	0.22	0.42	0.50	0.43
70	0	0	0	0.10	0.19	0.13	0.31	0.38	0.33
80	0	0	0	0.05	0.07	0.06	0.17	0.22	0.19
90	0	0	0	0	0	0	0	0	0

Table 6 : Conversion coefficients employed

Energy range (keV)	Direction of irradiation	Dose equivalent per unit air kerma (Sv Gy^{-1}) H_g	$H^*(10)$	$H'(10)$
10 - 60	AP	0.26	0.85	0.85
	PA	0.12	0.85	-
	ROT	0.13	0.85	0.33
60 - 180	AP	1.43	1.63	1.63
	PA	1.12	1.63	0.10
	LAT	0.69	1.63	0.95
	ROT	0.96	1.63	0.80
≥ 180	AP	1.07	1.21	1.21
(180 - 1500)	PA	0.93	1.21	0.18
	LAT	0.67	1.21	0.95
	ROT	0.82	1.21	0.80
V hard (a)	AP	1.02	1.13	1.13
>1000	PA	0.95	1.13	0.42
	LAT	0.77	1.13	0.99
	ROT	0.86	1.13	0.86

(a) Not used in the calculation of H_g, $H^*(10)$ and $H'(10)$.

Table 7 : Summary of results for various dose quantities

Organisation	Position of measurement in plant	Worker orientation	Estimated dose values (per unit air kerma) (Sv/Gy) H_g	$H^*(10)$	$H'(10)$
BNFL	1	AP	1.05	1.21	1.21
	2	AP	1.09	1.25	1.23
	3	LAT	0.67	1.25	0.95
	4	AP,PA	0.90	1.27	0.79
	5	AP,PA,LAT	0.92	1.26	0.73
	6,7,8,10,11,13,14,15	AP	1.07	1.21	1.21
	9	ROT	0.82	1.21	0.80
	12	AP,LAT,FH	0.89	1.21	1.07
Amersham International	1	AP	1.11	1.25	1.25
	2	AP	1.43	1.63	1.63
	3	AP	1.07	1.21	1.21
	4	AP	1.11	1.25	1.25
	5	AP	1.32	1.50	1.50
	6	AP	1.11	1.25	1.25
David Brown Gears	1	ROT	0 55	1.24	0.57
	2	ROT	0.84	1.27	0.80
	3	ROT	0 86	1.34	0.80
	4	ROT	0 90	1.46	0.80
	5	ROT	0 89	1.42	0.80
	6,7	ROT	0 92	1.50	0.80
	8,9	ROT	0 93	1.55	0.80
Gloucester Hospital	1	AP	1.11	1.26	1.26

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Contract no.: BI6-A-016-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A.F. McKinlay
National Radiological
Protection Board, NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Telephone number: 235-83.16.00

Title of the research contract:

Calculation of doses from external radiation.

List of projects:

1. Calculation of doses from external radiation.

Title of the project no.: 1

Calculation of organ doses from external photon irradiation

Head(s) of project:

Dr P J Dimbylow

Scientific staff:

Dr D G Jones

I. Objectives of the project:

To calculate organ doses and effective dose equivalent in various situations of practical dosimetric importance. Calculations should also be undertaken to assist in the understanding of experimental measurements. Particular areas covered are the calculations of medical exposures and the interpretation of the results of personal monitoring for epidemiological studies of working populations.

II. Objectives for the reporting period:

To calculate organ doses arising from a distributed plane source buried under a layer of soil and to compare the effective dose equivalent with a personal dosemeter reading.

III. Progress achieved:

Work on the comparison of personal dosemeter readings with organ doses and effective dose equivalent was extended to photon irradiation from an extended plane source, as would arise for example in a fallout situation.

The problem was suggested by G Williams (formerly of GSF) as a benchmark calculation at a EURADOS working group meeting, and consisted essentially of two parts:

a) To calculate the air kerma 1 m above ground due to an infinite plane Cs-137 source located in the soil. The soil composition was to be that used in a GSF publication (Jacob and Paretzke 1986).

b) To use the angular and energy distribution of the kerma to calculate the dose rate to the organs of a man standing in the field.

A program was written to calculate the mean kerma as a function of energy and angle, and an earlier program was modified to calculate the organ doses for each of a set of angular intervals. The source depth was set at 3 mm to enable direct comparison with the results published by GSF (Jacob et al 1988), and the organ doses were calculated for both the male and female GSF mathematical phantoms to give a composite effective dose equivalent.

The total air kerma was found to be identical to the GSF value (Jacob and Paretzke 1986) of 5.70E-16 Gy/sec per unit areal activity where areal activity is expressed as the number of source photons emitted per second from each square metre of the plane source. The effective dose equivalent was 4.08 $\pm$ .03E-16 Sv per photon per square metre compared with the GSF figure of 4.4E-16 (Jacob et al 1988). Most of the individual organ doses agreed with the GSF values to within 10%, and all but two to within 20%. The exceptions were the adrenals at 21 $\pm$ 5% lower, and the skeleton which was 22% higher. Some of the differences may arise because the present method treats the angular dependence of kerma by splitting direction space into a number of equal solid angles and then calculating the dose response for each segment. Whereas, the

GSF method uses data from 6 irradiation geometries and interpolates between them.

The effective dose equivalents for the separate male and female phantoms were 3.79 ± .04E-16 and 4.13 ± .05E-16 Sv per photon per square metre respectively. These values were compared with the doses recorded by an idealised dosemeter located on the midline of the anterior of the body 14 cm above the bottom of the trunk. The dosemeter on the male phantom gave 4.0 ± 0.2E-16, and that on the female 4.9 ± 0.3E-16 Sv per photon per square metre, ie, differences of 6% and 19% respectively, but in the male case of no statistical significance.

References

1) Jacob P and Paretzke H G 'Gamma-Ray Exposure from Contaminated Soil' Nucl. Sci. Eng., 93, 248-261 (1986).

2) Jacob P , Paretzke, H G, Rosenbaum H and Zankl M 'Organ Doses from Radionuclides on the Ground' Part 1, Simple Time Dependences. Health Phys. 54, No 6, 617-633 (1988).

IV. Objectives for the next reporting period:

To extend the calculations to other soils and to different source depths.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Attendance at EURADOS Committee 4 meeting 13-14th October at Cadarache, France.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board (NRPB)
Chilton, Didcot
GB Oxon OX11 ORQ

Contract no.: BI6-A-301-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A.F. McKinlay
National Radiological
Protection Board, NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Telephone number: 235-83.16.00

Title of the research contract:

Investigation of methods for improving the thermoluminescence properties of thin dosemeters for application to beta and low energy photon dosimetry.

List of projects:

1. Investigation of methods for improving the thermoluminescence properties of thin dosemeters for application to beta and low energy photon dosimetry.

Title of the project no.: 1

Investigation of methods for improving the thermoluminescence properties of thin dosemeters for specific application to beta and low energy photon dosimetry

Head(s) of project:

Dr C M H Driscoll

Scientific staff:

Mr T M Francis Mr J B O'Hagan Dr D T Bartlett

I. Objectives of the project:

i) To investigate the properties of thin thermoluminescent dosemeters suitable for use in beta dosimetry.

ii) To investigate methods for increasing sensitivity and lowering dose thresholds of these dosemeters, and

iii) To investigate methods for increasing the mechanical stability and rigidity of thin dosemeters for use in routine dosimetric applications.

II. Objectives for the reporting period:

i) To investigate the properties and preparational parameters of new high-sensitivity thermoluminescent materials.

ii) To investigate the incorporation of these materials into thin (or dosimetrically thin) dosemeters (eg, carbon-loaded detectors) for use in beta dosimetry, and

iii) To investigate instrumental methods for improving the detection threshold.

III. Progress achieved:

Research on the dosimetric properties of LiF/PTFE discs loaded with various carbon concentrations has continued. The responses of these dosemeters as a function of radiation quality and angle of incidence are generally acceptable for personal and extremity dosimetry applications. However, the detection threshold is high (~ 1 mSv). Two approaches used to lower the detection threshold are (a) increase the amount of light emitted for a given absorbed dose and (b) increase the light collection or recording ability of the readout system.

One way of achieving the first approach is to use a higher sensitivity thermoluminescent material. LiF:Mg,Cu,P powder (GR-200P) produced by the Research Institute of Chemical Defence of China has a reported sensitivity of between 30 and 60 times that of TLD-100 (LiF:Mg,Ti). The manufacturers recommend that in order to maintain the high sensitivity the material should not be annealed above 245°C. This limits its use. For example, the material could not be incorporated in PTFE discs without loss of sensitivity since fabrication of these discs requires thermal treatments at 360°C. In order to overcome this difficulty, experiments have been carried out on thin dosemeters incorporating a layer of GR-200P powder (~ 3 mg, grain size 40-70 μm) onto Kapton heat resistant adhesive tape. These dosemeters are in the form of discs approximately 12 mm diameter. The maximum readout temperature for these dosemeters is 240°C. The beta response ratios (promethium-147 to strontium-90/yttrium-90) for both the new discs and for carbon-loaded LiF/PTFE discs were 0.6. However, the detection thresholds for the new discs were lower than for the carbon-loaded dosemeters. At the 95% confidence level, the detection thresholds for the two types of dosemeter were 30 μSv and 1 mSv, respectively. The only major problem with the new dosemeters in their present form is their lack of rigidity. Additional layers of Kapton tape can be used on the dosemeter surface to increase rigidity. However, longer heating periods are required during readout of these thicker dosemeters when using contact heating. More efficient methods of heating such as hot gas or radiant infra-red should alleviate this problem.

Although LiF:Mg,Cu,P/Kapton discs have potential for use in beta dosimetry, a more suitable dosemeter for routine application would be LiF:Mg,Cu,P incorporated in a carbon-loaded PTFE disc. Since the sensitivity of LiF:Mg,Cu,P is high, a decrease in sensitivity, as a result of thermal treatment above 245°C, may still produce a dosemeter with an acceptable detection threshold. Therefore, a study is in progress to quantify the effect on the sensitivity of LiF:Mg,Cu,P powder of thermal treatments at 360°C (the temperature required to produce PTFE discs). The initial results suggest that a short anneal produces a decrease in sensitivity of 50% which is reproducible. Providing no other anneal treatment above 245°C is used, the sensitivity remains at this level. Further studies are required to determine if a number of discs can be manufactured using LiF:Mg,Cu,P powder and a carbon/PTFE matrix. These dosemeters should then have dosimetric properties similar to those of the LiF:Mg,Ti/PTFE carbon loaded discs, but with an improved detection threshold.

A pilot study was carried out to produce a batch of standard thermoluminescent grade lithium fluoride doped with magnesium titanium (LiF:Mg,Ti) as a precursor to the incorporation of other impurities to

produce our own high sensitivity. The reasons, yet to be resolved, may be related to inefficiencies in the quenching or doping stages.

As part of the study to find instrumental methods for improving detection thresholds, the technique of glow curve deconvolution has been investigated. This technique is already used in a number of commercial TLD reader systems. The background and radiation induced signals can be separated using fitting techniques to identify the characteristic glow curve shape for materials such as LiF:Mg,Ti. However, these techniques require an identifiable glow curve, which is often difficult to distinguish from background for low absorbed doses (less than 500 μGy). A prototype system has been developed which records the time of each reader output pulse and then uses the inverse of the interval between pulses to determine the frequency of pulse emission, and hence the glow curve. Initial studies suggest that reasonable glow curves are produced after exposure times of a few hours to natural background radiation for LiF:Mg,Ti (TLD-100 chips). Further studies are required to incorporate such a system into the front end of a glow curve deconvolution procedure.

A study has been carried out to determine the dose verification characteristics for the commercial extremity dosemeter manufactured by Vinten Instruments Ltd. A successful regime has been derived and is to be published. The technique will also be explored for the carbon-loaded dosemeters.

IV. Objectives for the next reporting period:

i) To incorporate high sensitivity LiF:Mg,Cu,P into carbon-loaded PTFE disc dosemeters.

ii) To study the angular and field dependence on such dosemeters in mixed beta-gamma fields.

iii) To study the technique of glow curve deconvolution for analysis of data close to the detection threshold.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None.

VI. Publications:

O'Hagan J B, Francis T M, Williams S M 'The Re-usability of the Vinten Extremity Dosemeter', Radiation Protection Dosimetry 20, 3 (1987).

Francis T M, O'Hagan J B, Williams S M, Driscoll C M H, Bartlett D T 'Response Characteristics of Carbon-Loaded TL Detectors to Beta Radiation', to be published in Radiation Protection Dosimetry.

O'Hagan J B, Francis T M, Williams S M, Driscoll C M H, Bartlett D T 'The Response of Carbon-Loaded LiF/PTFE Thermoluminescent Detectors to Beta and Low-Energy Photon Radiation at Various Angles', to be submitted to Radiation Protection Dosimetry.

O'Hagan J B, Pearson A J, Dutt J C 'Re-assessment of Dose from the Vinten Extremity Dosemeter', to be submitted to Radiation Protection Dosimetry.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Universität des Saarlandes
St.Johanner Stadtwald
D-6600 Saarbrücken

Contract no.: BI6-A-010-D

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H.G. Menzel
Universität des Saarlandes
Boris Rajewsky Institut
D-6650 Homburg (Saar)

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Universität des Saarlandes
Boris Rajewsky Institut
D-6650 Homburg (Saar)

Telephone number: 6841-16.62.02

Title of the research contract:

Dosimetric research and radiation protection dosimetry with proportional counters and physical and biological accident dosimetry.

List of projects:

1. Basic physical data for the dosimetry and radiation protection dosimetry of neutrons and photons with low pressure proportional counters.
2. Investigation of practical aspects of employing microdosimetric counters as dose equivalent meters.

Title of the project no.: 1

Basic physical data for the dosimetry and radiation protection dosimetry of neutrons and photons with low pressure proportional counters

Head(s) of project:

H. G. Menzel

Scientific staff:

P. Pihet
K. H. Folkerts
P. Dahmen

I. Objectives of the project:

The accuracy and precision achievable in dosimetry of external ionising radiation depends on the availability of adequate basic physical data. Examples for required improvements are neutron interaction data (cross sections, secondary charged particle spectra) and dosimetric data (kerma factors) for different elements and materials and neutron energies above 14 MeV. The ability of low pressure proportional counters (PC) to measure absorbed dose with relatively low uncertainty, and simultaneously ionisation events events due to single primary interactions are used to determine required interaction and dosimetric data for neutrons and photons.

II. Objectives for the reporting period:

To perform combined pulse height and time-of-flight measurements with low pressure proportional counters with walls made of A-150 plastic and graphite in beams of quasi-monoenergetic neutrons in the energy range 20 - 60 MeV (at Paul Scherrer Institute, PSI (formely SIN), Switzerland). To evaluate ionisation yield spectra and kerma (ratios) for different energies. To continue to investigate the potentials of the combination of A-150 and graphite counters ("twin counters") to evaluate data relevant to the application of the cavity chamber principle to high energy neutrons.

III. Progress achieved:

Work within this project has been focussed on the improvement of the accuracy achievable in kerma measurements for fast neutrons with low pressure proportional counters. Improvements of the calibration procedures with the usually built-in alpha particle source were achieved using the so-called proton and alpha particle edges. The overall uncertainty of the measurements depends critically on the quality of the basic physical data required in the application of the cavity chamber principle, in particular W-values, stopping powers and stopping power ratios for the charged particles released and their energy dependence. Measurements previously carried out with tissue equivalent (TEPC) and carbon walled (CPC) proportional counters with monoenergetic neutrons in the range of 5 to 60 MeV have been used to investigate possibilities to reduce uncertainties in the evaluation of kerma. The method used takes advantage of the ionisation yield spectra provided by the proportional counters. The investigation included a thorough research of published data for W-values and stopping powers.

The approach taken is based on the principal possibility to distinguish different events due to different charged particles within the measured ionisation yield spectra. The limitation of the corresponding unfolding into absorbed dose contributions by protons, alpha particles and heavier ions is due to the overlapping of the event sizes within the spectra and has been partly overcome by combined analysis of TEPC and CPC measurements under identical conditions and by using calculated energy deposition spectra. Recoil protons and heavier particle events were discriminated by appropriately fitting the spectra measured with the CPC above 150 keV/µm to the corresponding TEPC spectra. The underlying and justified assumption is that the interactions with the small oxygen, nitrogen, calcium and fluorine components in A-150 plastic can be approximated by carbon interactions. The different charged particle dose components due to carbon interactions (protons, alpha particles, C, Be and B ions) were separated using calculated ionisation yield spectra. The partial contributions obtained by this method were used to assess the effective energies of individual types of particles entering the cavity and subsequently effective stopping power ratios and effective W-values were evaluated. In order to obtain effective gas-to-wall absorbed dose conversion factors the contribution of primary interactions of the neutrons with the gas in the cavity were also taken into account.

The method applied confirmed the advantages of low pressure proportional counters for kerma measurements and critical evaluation of the results due to the low mass of the gas in the cavity and the ionisation being measured for single primary particles. With the help of recent precise data on W-values for protons and alpha particles by Thomas and Burke (Phys.Med.Biol.30, 1201-1223 (1985)) the uncertainties of the effective W-values W_n for neutrons with energies up to 60 MeV could be reduced to 1.5%-2.5%. In Figure 1 the results for W_n in TE-chambers are compared to data by Bichsel.

The values obtained for gas-to-wall conversion factors as function of neutron energy differ considerably from those published by Makarewicz et al. (Phys.Med.Biol.31, 281-284 (1986)) differ considerably due to differences in the application of the Bragg additivity rule. Makarewicz et al. used mean excitation energies for TE-gas and material whereas in our

method stopping power data for elements were added according to the Bragg rule. At 15 MeV the corresponding differences in the conversion factors are 1.7% for the TEPC and 8% for the CPC.

The paucity of data for W-values in propane based TE-gas turned is a serious limitation for the accuracy achievable with this mixture frequently used in microdosimetry. The proportionality between W-values for propane and methane TE gases implicitly assumed by many authors does not appear to be valid for charged particle energies below 500 keV.

The improved data for W-values and stopping power ratios have been used to re-evaluate former kerma factor measurements. The differences between original and revised results for monoenergetic neutrons between 14 and 19 MeV were up to 3%. For radiation fields with broad neutron energy spectra the differences were considerably larger.

The planned combined pulse height and time-of-flight measurements with TEPC and CPC for monoenergetic neutrons above 20 MeV had to be postponed to early 1989 due to lack of irradiation time.

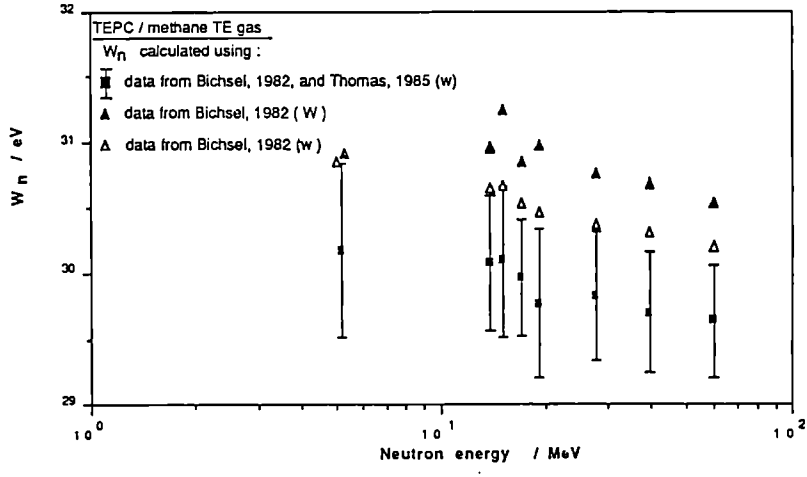

Figure 1: Average W-values for monoenergetic neutron beams from 5 to 60 MeV derived for a cavity filled with methane based TE-gas (diameter 0.1 mg/cm2). Effective mean energies were assessed from ionisation yield spectra measured with TEPCs and CPCs filled with propane TE-gas. Integral and differential W-values were calculated using the W-function given by Bichsel and Rubach (Phys. Med. Biol., 27, 1003-1013 (1982)). Final results were obtained using the W-values for protons above 1 MeV by Thomas and Burke. Overall uncertainties are indicated.

IV. Objectives for the next reporting period:

To perform pulse height and time-of-flight measurements with low pressure proportional counters (PC) with walls made of A-150 and graphite in beams of quasi-monoenergetic neutrons (30 - 40 MeV) in combination with neutron fluence measure-ments performed by PTB. To evaluate ionisation yield spectra and kerma factors for A-150 and carbon for these energies. To compare the results with theoretical data based on nuc-lear model calculations.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Physikalisch Technische Bundesanstalt (PTB), Braunschweig, Gruppe Neutronendosimetrie (Prof. G. Dietze)
Université Catholique de Louvain, Louvain-la-Neuve, Belgium (Prof. J. P. Meulders and A. Wambersie)
National Institute for Science and Technology (NIST), Washington,D.C., Center for Radiation Research (Dr.J.J. Coyne, Dr.R.S. Caswell)

VI. Publications:

Menzel, H. G.,Pihet, P.,Folkerts, K. H.,Grillmaier, R. E., Dosimetry research using low pressure proportional counters for neutrons with energies up to 60 MeV, *Radiat. Prot. Dosim.* 23, 389 - 392 (1988)

Pihet, P., Gueulette, J., Menzel, H. G., Grillmaier, R. E., Wambersie, A., Use of microdosimetric data of clinical relevance in neutron therapy planning, *Radiat. Prot. Dosim.* 23, 471 - 474 (1988)

Menzel, H. G., The use of low pressure proportional counters in neutron dosimetry, in Ionizing Radiation: Protection and Dosimetry, G. Paic, ed., CRC Press Boca Raton, 187 - 216 (1988)

Schrewe, U. J., Brede, H., Pihet, P., Menzel, H. G., Improvements on the calibration of tissue-equivalent proportional counters with built-in α-particle sources, *Radiat. Prot. Dosim.*, 23, 249 - 252 (1988)

Title of the project no.: 2

Investigation of practical aspects of employing microdosimetric counters as dose equivalent meters

Head(s) of project: H. G. Menzel

Scientific staff:

A. Kunz
P. Pihet
P. Dahmen
K. H. Folkerts

I. Objectives of the project:

Radiation protection dosemeters based on tissue equivalent proportional counters (TEPC) have been shown to have good dose equivalent response for neutrons in the energy range from thermal neutrons to 20 MeV and to possess diagnostic capacity for mixed radiations. The construction of a portable area monitor based on a TEPC will be completed and the instrument will be tested in reference neutron and photon fields and mixed radiations fields in environments of practical importance. The construction will be optimised for operational health physics requirements and with regard to dose equivalent response.

II. Objectives for the reporting period:

To carry out the final evaluation of the second part of the EURADOS intercomparison of TEPC instruments at PTB and of the TEPC measurements with small ^{3}He-gas additions to the TE-gas mixture. To use these results to optimize the dose equivalent response of the TEPC and the calibration and operation procedures of TEPC based area monitors. To continue radiation protection measurements with TEPC instruments in mixed radiation fields of practical interest. To continue work on the development of the portable TEPC area monitor suitable for operational health physics and to test its performance.

III. Progress achieved:

The prototype version of our area dosemeter "HANDI" based on a tissue equivalent proportional counter (TEPC) has been finalized. All components were newly developed by CAD-techniques, are suited for semi-industrial production and are designed in such a way that surface mounted devices (SMD) may be used in future. This technique would enable the reduction of the total size of the electronic components by a factor of four. The present version is mounted for convenience inside a 19 inch rack and is operated by four control push buttons. The weight is 6 kg (without detector) of which 2.5 kg is the NiCd battery pack. If required, detector and electronics may be separated by a distance of up to 50 m. The maximum power consumption is 5 W at +12 V and ±5 V. Operation times of 40 hours can be achieved with the incorporated battery pack. The next version will use a new CMOS single-board computer which will further reduce the power consumption and simplify the digital electronics. With this technique the construction of a real hand held area dosemeter will be possible. The final optimisation of software and operation procedures will be performed using the new version.

The measurements with the HANDI area dosemeter and a conventional microdosimetric laboratory system (named BIO) at Physikalisch Technische Bundesanstalt (PTB) within the second part of the EURADOS intercomparison of TEPC area dosemeters were evaluated. The average ratio of the dose equivalent responses of BIO and HANDI is 0.94 with a standard deviation of 0.07. For absorbed dose the ratio is 1.01 ±0.07. This agreement confirms the suitablility of the 16-channel method applied in the HANDI prototype. Comparisons of the spectra also document the good agreement between the results of the conventional technique and the prototype operational instrument. Further details of the intercomparison can be found in the progress report of EURADOS working group No. 1 and in a report by PTB (PTB-FMRB-177, 1988).

The effects of adding small amounts of ^{3}He to the tissue-equivalent gas mixture of a TEPC and of varying in addition the wall thickness was experimentally investigated for monoenergetic neutrons (thermal neutrons, 24 keV, 144 keV and 2.5 MeV neutrons) and in the radiation field of a D_2O moderated ^{252}Cf-source at PTB. This approach to improve the too low dose equivalent response of TEPC in the low and intermediate neutron energy range takes advantage of the moderation and thermalisation of low energy neutrons in the counter wall, the large cross-section (5300 barn) of the $^{3}He(n,p)^{3}H$ reaction for thermal neutrons and the large kinetic energy of the proton and tritium recoil released in the reaction. The drastic effect of this reaction on the microdosimetric spectrum for thermal neutrons is illustrated in Figure 2. In this measurement only 0.1% ^{3}He has been added to the counting gas. The results of the investigation show that addition of small amounts of ^{3}He increases the dose equivalent response of thin walled TEPC's for neutron energies below 200 keV without deteriorating their response at higher energies. However, addition of 1% ^{3}He is not sufficient to compensate the too low response at energies below 100 keV. Increasing the wall thickness and thus the fraction of thermalized neutrons leads to a significant increase in the dose equivalent response for 24 keV neutrons. Although a final conclusion of the optimum combination of wall thickness and ^{3}He addition to the gas requires more data and should include dedicated calculations it appears that for the counters used a wall thickness of approximately 5 mm and a ^{3}He content

of 2% of the TE gas mixture may result in a considerable improvement of the dose equivalent response.

The results obtained have shown that the ^{3}He addition also enables quantitative assessments to be made of the neutron thermalisation in the counter wall. This additional possibility of the approach with ^{3}He increases the range of applications of TEPC. For example, at the D_2O moderated ^{252}Cf source a 2% contribution of thermal neutrons to total primary fluence was determined. For a wall thickness of 17 mm the contribution of thermal neutrons increased to 25%. Measurements with ^{3}He-TE gas mixtures may prove to be very useful in combination with neutron transport and energy deposition calculations.

Our measurements in low energy neutron reference fields were continued at the filtered reactor beams at National Bureau of Standards (NBS, now NIST) in Washington, USA. The 2 keV and 24 keV beams are very useful for investigations with TEPC's because of the relatively low contamination with neutrons of higher energies. The first evaluation has confirmed the good quality of the irradiation beams. The final evaluation is being carried out at present.

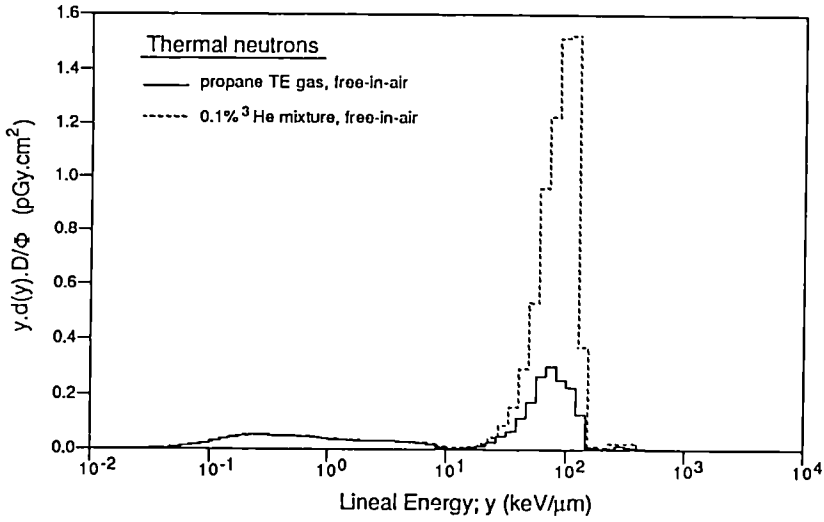

Figure 2: Fluence normalised dose distributions in lineal energy y for thermal neutrons measured with TEPCs with and without addition of 0.1 % ^{3}He gas to the propane TE-mixture. The total area under the curves is equal to the total absorbed dose per incoming neutron.

IV. Objectives for the next reporting period:

To carry out further measurements with the portable prototype TEPC area dosemeter in radiation environments of practical interest. To implement the established optimisation procedures with regard to calibration, operation, neutron-photon discrimination in the hardware and software of the area dosemeter. To complete the study of the influence of ^{3}He-gas additions and wall thickness on the dose equivalent response. To build several identical TEPC area dosemeters to be used by operational health physic at different institutes.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

EURADOS Working Group I
Physikalisch Technische Bundesanstalt (PTB), Braunschweig
(Profs. W. Alberts and G. Dietze)
Université Catholique de Louvain, Louvain-la-Neuve (Belg.), (Profs. J. P. Meulders and A. Wambersie)

VI. Publications:

Menzel, H.G., Dietze, G., Schuhmacher, H., Practical determi-nation of dose equivalent using low pressure proportional counters, in "Radiation Protection Practice", *Proc. of. 7th Int. Congress of the Int. Radiation Protection Asscociation, Sydney*, April 1988, S. 308 - 311, Pergamon Press (1988).

Folkerts, K. H., Menzel, H. G., Schuhmacher, H., Arend, E., TEPC radiation protection dosimetry in the environment of accelerators and at nuclear facilities, *Radiat. Prot. Dosim.* 23, 261 - 264 (1988)

Vynckier, S., Sabattier, R., Kunz, A., Menzel, H. G., Wambersie, A., Determination of dose equivalent and the quality factor in the environment of clinical neutron beams, *Radiat. Prot. Dosim.* 23, 269 - 272 (1988)

Dietze, G., Booz, J., Edwards, A. A., Guldbakke, S., Kluge, H., Leroux, J. B., Lindborg, L., Menzel, H. G., Nguyen, V.D., Schmitz, Th., Schuhmacher, H., Intercomparison of dose equivalent meters based on microdosimetric techniques, *Radiat. Prot. Dosim.* 23, 227 - 234 (1988)

Menzel, H. G., Schuhmacher, H., Cartier, F., Radiation pro-tection dosimetry of neutrons and photons at a high energy accelerator using a low pressure proportional counter, IV. European Congress of IRPA, Compact, p. 597 - 602 (1988)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Polytechnic of the South Bank
Borough Road
GB London SE1 OAA

Contract no.: BI6-A-025-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A.C. Norris
Dept of Phys.Sc.& Techn.
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Dr. A.K.M.M. Haque
Dept of Phys.Sc.& Techn.
Polytechnic of the South Bank
Borough Road
GB London SE1 OAA

Telephone number: 1-928.89.89

Title of the research contract:

Computation of the radiation dose due to the daughter products of radon deposited in the lung.

List of projects:

1. Computation of the radiation dose due to the daughter products of radon deposited in the lung.

Title of the project no.:

BI6-025-UK

Computation of the radation dose due to the daughter products of radon depsited in the lung.

Head(s) of project:

Dr. A.K.M.M. Haque

Scientific staff:

Dr. I.A.M. Al-Affan

Research Fellow

I. Objectives of the project:

Human exposure to radon and its daughter products, even in the normal living and working conditions, could amount to a substantial proportion of the total exposure to natural radiation. A comprehensive computation of the radiation dose to the lungs would be valuable in view of the new information on aerosols, lung parameters, stopping power, etc.

II. Objectives for the reporting period:

Lung Cancer induction model with information on lung cells, their radiosensitivity and distribution

Sensitivity analysis and risk evaluation with epidemiological and radiobiological information.

Preliminary study of Monte Carlo simulation in the dosimetry of the lung.

III. Progress achieved:

Local energy deposited in a sensitive site of 1 μm diameter, has been calculated for alpha particles, emitted from radon daughters lining the mucus layer in the respiratory tracts. The main alpha emitters are RaA (Po-218) and RaC' (Po-214) with alpha energies of 6 and 7.7 MeV, respectively. This calculation has then been followed by the dose evaluation and estimation of mutation (using data of Thacker et al. Radiat. Res., 92, 343, 1982) and transformation (using Hieber et al. Int. J. Radiat. Biol., 52, 859, 1987) of lung cells as a function of age. Mean life span of the stem cells was varied between 5 and 45 years to simulate the living condition in different environments.

Figures 1 and 2 give the results of the estimation of mutation and transformation, respectively, for radon concentration of 23 Bq/m^3 (Average in UK dwellings). Similar calculations have been carried out for other concentrations up to 230 Bq/m^3. Also, the cumulative fraction of transformed cells after 40 and 70 years has been calculated for radon concentration in the range 23-230 Bq/m^3 (figure 3). Increase of the fraction of transformed cells with the radon concentration was exponential.

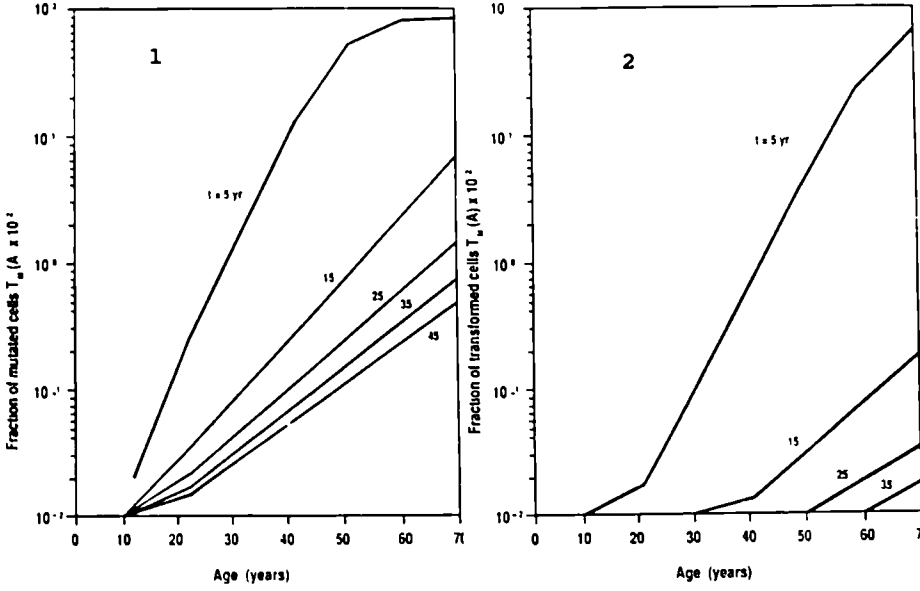


Fig. 1. The fraction of mutated cells vs. age for different life span, t.

Fig. 2. The fraction of transformed cells vs. age for different life span, t.

It is concluded that alpha particles may initiate the transformation in the stem cells, but the appearance of lung cancer may depend strongly on the promotion of other factors such as smoking.

On comparison of female lung cancer deaths (OPCS figures) during 1951-60 in Devon and Cornwall, average radon concentrations of 74 and 110 Bq/m^3 (NRPB Survey, 1987), it has been concluded that the risk due to radon exposure alone is 60 per 10^6 per year per WLM, while the baseline risk due to causes other than radon is 40 per 10^6 per year.

We have also examined the total lung cancer deaths of males and females in Cornwall and Devon during 1961-1970 and 1971-1985 using ICRP Task Group (1987) derivation of the attributable excess lifetime risk as a function of indoor exposure to radon for the mixed population of smokers and non-smokers.

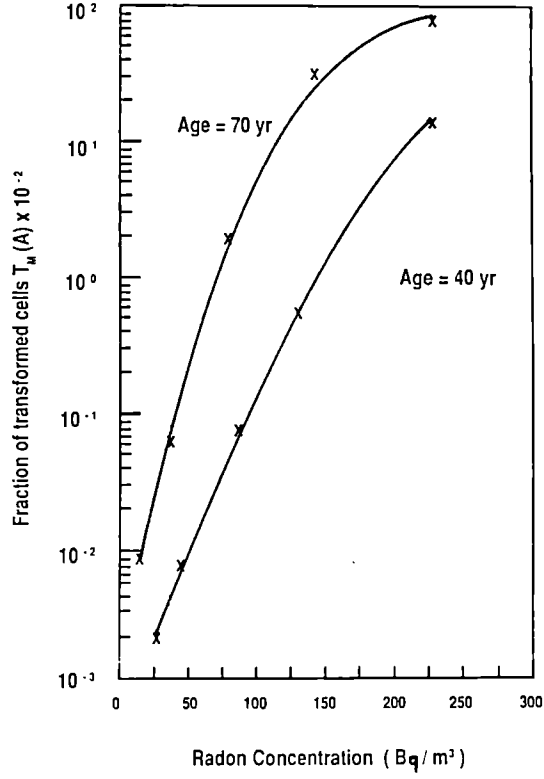


Fig. 3. The fraction of transformed cells vs. radon concentration for cumulative exposure over 40 and 70 years, t=45yr.

IV. Objectives for the next reporting period:

1) Estimate the fraction of transformed cells using specific energy, z, distribution instead of the average dose, D, taking into account that cells may be hit more than once per year.

2) Study of Monte Carlo simulation in the dosimetry of the lung.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. D.M. Taylor University of Heidelberg, Germany.

Dr. J. Miles NRPB, UK.

VI. Publications:

1) HAQUE, A.K.M.M., and AL-AFFAN, I.A.M. (1988). Main factors affecting the calculation of radiation dose to the lung from inhalation of radon daughters. The Science of the Total Environment, 74, 279-288.

2) AL-AFFAN, I.A.M. and HAQUE, A.K.M.M. (1989). Local energy deposited for alpha particles emitted from inhaled radon daughters. Physics in Medicine and Biology, 34,97-105.

3) HAQUE, A.K.M.M., and AL-AFFAN, I.A.M. (1988). Exposure to high environmental radon concentration in the UK-Lung cancer risk?. Poster presented at the 14th L.H. Gray Conference, Oxford, 11-15 Sept. 1988.

4) AL-AFFAN, I.A.M. and HAQUE, A.K.M.M. (1989). Mutation and transformation of lung cells from inhalation of radon daughters in dwellings: A preliminary study. Int. J. Radiat. Biol. (submitted).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Contract no.: BI6-A-018-UK

Head(s) of research team(s) [name(s) and address(es)]:

Mr M.C. O'Riordan
Radiological Measurement Dept
NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Telephone number: 235-83.16.00

Title of the research contract:

The development of realistic phantoms to assist in the interpretation of *in vivo* measurement of low-energy photon-emitting radionuclides in bone.

List of projects:

1. Production of skull and chest phantoms suitable for the calibration of detectors for the measurement of 241-Am, 210-Pb and 90-Sr in bone.

Title of the project no.:

1

Production of skull and chest phantoms suitable for the calibration of detectors for the measurement of ^{241}Am, ^{210}Pb and ^{90}Sr in bone.

Head(s) of project:

F A Fry

Scientific staff:

M R Bailey, A Birchall, N J Dodd, M D Dorrian, G Etherington, N Green, J D Harrison, J R H Smith.

I. Objectives of the project:

The objective of this project is to design and construct phantoms which can be used to calibrate detectors for in vivo measurement of low-energy photon-emitting radionuclides in bone.

II. Objectives for the reporting period:

Further longitudinal scans will be carried out on subjects injected with bone-seeking radionuclides, and inferences drawn about the skeletal distributions. It is expected that another subject will receive ^{88}Y, and two more ^{239}Np.

In view of the overall objective of improving techniques to assess the body content of bone-seeking radionuclides, a model of plutonium retention and excretion will be developed.

Experiments will be conducted on the application of radionuclides to bones in a controlled manner. Artificial substitutes for overlying and underlying tissues will be prepared. Skulls will be obtained and labelled.

III. Progress achieved:

(i) Measurements of the distribution of bone-seeking radionuclides in the body

Advantage was taken of further opportunities to measure the distribution of bone-seeking radionuclides in man, resulting from the administration of ^{88}Y ($t_{1/2}$ 107 d) and ^{239}Np ($t_{1/2}$ 2.4 d) to human volunteers in studies of radionuclide biokinetics conducted at NRPB. The distribution of activity in the body was measured using the longitudinal scan technique described in the last report.

A second subject was intravenously injected with 4 kBq ^{88}Y. Profile scans on the previous subject, which had been made at 2 hours, 22 days and 84 days after intake, indicated that redistribution of the activity in the body occurred predominantly between the first two measurements. Scans were therefore made on the second subject at 5, 25 and 49 hours and at 4, 7, 9, 15 and 24 days after injection. These measurements showed that the distribution of activity changed steadily over the first four days, after which it remained approximately constant and consistent with the distribution of bone surface area. The activity distributions measured in the first and the last of the scans made on this subject were very similar to those measured on the previous subject at corresponding times, which were described in detail in the last report. Therefore it appears that intravenously administered ^{88}Y, which on the basis of animal experiments is expected to be widely distributed in the body initially, is deposited on bone surfaces over a period of about four days.

A second human volunteer was injected with 2.5 kBq ^{239}Np, and a longitudinal scan was made 1 day after injection. The results were very similar to those for the previous subject (described in detail in the last report), again being consistent with the bone surface distribution.

(ii) Biokinetic model for plutonium

Because of the low yields and energies of photons emitted in the decay of plutonium isotopes, they cannot be measured directly in the human body at levels corresponding to annual limits on intake. Evaluation of intakes may have to rely on air sampling or excreta monitoring or on a combination of techniques. A major problem with interpreting excreta measurements is the lack of a satisfactory biokinetic model. Models developed for calculating doses from intakes do not represent excretion sufficiently accurately, while mechanistic models which do so are too complicated for routine practical use.

Existing models and relevant information on plutonium biokinetics in man were revieved. A model is being developed that is simple, but soundly based biologically, and which adequately represents the retention in body tissues and urinary and faecal excretion rates of plutonium following systemic uptake in man. The model has two bone compartments, two liver compartments, and two tissue compartments, all linked to a central blood compartment by first order kinetics. The parameters linking the bone compartments were obtained by reducing a complex metabolic model of bone surface-seeking radionuclides to a

simpler yet mathematically equivalent system. The remaining parameters in the model are derived from observed urine and faecal rates of plutonium measured in man in the study conducted by Langham.

(iii) Bone phantoms

Recent legislation in India and the United Kingdom has severely decreased the availability of natural human bones. However, a commercial supplier has now been found who expects to be able to meet our requirements. The skull was identified as the best site to measure for estimating the activity in the skeleton, because of the presence of a large mass of bone which is relatively independent of body size and is near the surface, and minimal interference from other organs. For each radionuclide and subject type, a matched pair of skulls is required, one labelled on the inside, the other on the outside. The response of a detector to the required distribution of activity in the skull will be obtained by appropriate weighting of its response to the two phantoms. Thorax phantoms are required in order to make allowance for activity in the bones of the chest when measuring activity in the lungs. An order has been placed for twelve skulls, three matched pairs of adult male skulls, one pair adult female, one pair preadolescent (8-12 y), and one pair infant (9-18 months). The first seven vertebrae will accompany each skull. The possibility of obtaining suitable thorax skeletons is being investigated.

Skull phantoms labelled with several radionuclides, and a thorax phantom labelled with ^{241}Am have been made at New York University Medical Center (NYU). The United States Transuranium Registry also has an ^{241}Am phantom, constructed with bones from a subject who was internally contaminated in life. Discussions were held with Dr N Cohen at NYU and Dr H E Palmer at Battelle Northwest Laboratories, at which it was confirmed that these phantoms could be borrowed for short periods. Arrangements are being made to borrow from NYU skull phantoms labelled with ^{210}Pb and ^{241}Am, and the ^{241}Am thorax phantom during the coming year. A programme of measurements is being planned which for the ^{241}Am phantom will include measurements at other establishments in the UK with whole-body monitoring facilities.

IV. Objectives for the next reporting period:

Further longitudinal scans will be carried out on subjects injected with bone-seeking radionuclides, and inferences drawn about the skeletal distributions. Measurements will continue on the subject who received ^{88}Y and it is expected that two more will receive ^{239}Np. Development of the plutonium biokinetic model will continue. Experiments will be conducted on the application of radionuclides to bones in a controlled manner. Artificial substitutes for overlying and underlying tissues will be prepared. Skulls will be obtained and labelled.
Measurements will be made on the NYU ^{241}Am and ^{210}Pb phantoms using both dual-phosphor and germanium detectors, and comparisons will be made with phantoms constructed here.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P.N° 6
F-92260 Fontenay-aux-Roses

Contract no.: BI6-A-020-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. G. Portal
IPSN/DPT/SIDR
CEA - CEN, Fontenay-aux-Roses
B.P.N° 6
F-92260 Fontenay-aux-Roses

Telephone number: 46.54.72.28

Title of the research contract:

Neutron individual and area dosimetry, realization of neutron calibration sources, beta-particle dosimetry, accident dosimetry of clothes.

List of projects:

1. Estimation of mean electron energy.
2. Thermoluminescence and exoelectron dosimetry in tissue of cotton.
3. Dosimetry on natural and synthetic tissue by ESR.
4. Realization of an "operational" spectrometry unit for neutrons.
5. Study and realization of an individual dosimeter based on photographic emulsions.
6. Area and individual dosimetry with proportional counters.

Title of the project no.: 1

Estimation of mean electron energy

Head(s) of project:

J. BARTHE - M. PETEL

Scientific staff:

R. CHUITON - L. LE CORRE

I. Objectives of the project:

The method is based on the use of a single dosemeter which can be read either simultaneously or consecutively by radiothermoluminescence (RTL) and thermally stimulated exoelectron emission (TSEE). The difference between the responses obtained using these two techniques arises from differences between optical transmission through a depth of a few hundred micrometers in the case of RTL and electron transmission from a depth of between 1 and 10 nm from the detector surface in the case of TSEE.

II. Objectives for the reporting period:

Several objectives were covered during this reporting period :

a) The completion of the development of the new TSEE reader head (multineedle)

b) Completion of the electronics for separating TSEE and TL signals.

c) Applications to measurements of the mean energies of composite β radiations. The TSEE signal reproducibility of the new batches of beryllium oxide have caused some difficulty in these last measurements.

III. Progress achieved:

Large variations in sensitivity and reproducibility have been observeed with the most recent batches of beryllium oxide ceramics. No such variations occurred with the first batch.

The pellets were subjected to the treatment recommended by several workers 1000°C for 12 to 15 hours before being used for the first time ; 15 minutes at 400°C between successive uses with rapide cooling (TL treatment).

The thermostimulated exoelectron emission TSEE measurements exhibited the largest amount of variation in reproducibility with a standard deviation σ of about 25% for a signal to noise ratio R = 3 ; a better reproducibility was observed with the thermoluminescence measurements, $\sigma \neq 5\%$ for an R value of 10 with irradiation doses of 10 mGy.

No significant variations in the temperatures of the peaks measured were associated with these fluctuations.

It is recalled that the first results obtained using this method were very encouraging. These latest results have led us to question the usefulness of the work being performed.

An intermediate solution consisting of depositing at high temperature a thin layer (a few microns thick) of beryllium oxide on the ceramic surface has now been envisaged.

This should ensure that the detector simultaneously possesses the characteristics of a thin film as far as TSEE is concerned R = 100, $\sigma \neq 5\%$ as well as those of a pellet for TL measurements R = 10, $\sigma \neq 5\%$ for a dose of 10 mGy.

TSEE and TL sensitivities should be optimized. Different manufacturers have been consulted for the fabrication of these pellets.

Separate TL and TSEE measurements have been made so as to be able to continue studies on the design of a series model double-headed reader

TL/TSEE detection

Difficulties have occurred in attempting to distinguish photons emitted from the surface of the detector due to TL from those resulting from crown discharge associated with electron multiplication.

Several pertinent aspects of the problem are :

a) the discharge photons are generated in a region very close to the anode (needle).
b) the purity of the circulating gas (methane) is an important factor. Gain increases when argon is added ; this is, however, accompanied by an increase in the number of optical de-excitations
c) it is essential to use a photon counting system in order to distinguish between small amplitude TL pulses and TSEE pulses (10 to 1000 greater depending on the VHT applied)
d) in both cases, a very significant small amplitude component occurs ; discrimination cannot therefore be employed
e) background noise however behaves very differently : no background occurs with TSEE measuements, the TL background signal remains constant from room temperature up to about 600°C. Beyond 600°C a simultaneous growth occurs in both TSEE and TL signals, the kinetics being quite similar (wide band interference filter, 376 nm).

A schema of the series production head currently being manufactured is shown in figure (1). It is seen that a silica window delimits the methane filled volume.

This window is lightly metalized in order to create an electrical equipotential without any photon absorption.

The multineedle cell possesses 7 needles, compromising between the collecting solid angle for the photons and the total effect of the electric field lines.

Composite β radiation

Composite beta radiation is obtained by successively irradiating a dosemeter using a stontium-90/yttrium-90 source and promethium-147 source.

The following dose composition ratios were employed :

Sr-90/Y-90 0 2 4 6 8 10

Pm-137 10 8 6 4 2 0

A total constant dose of 10 mGy is deposited in each cose.

Signal normalization is performed with the Sr/Y source.

The first estimations give within the limits of experimental error (20 to 30%) a linear relation between the TL/EETS signal ratio and the Pm-147 dose.

Conclusion

Work on mean energy determinations is almost complete. An uncertainty however persists as for as the reliability of beryllium oxide ceramics is concerned. More exhaustive measurements are currently being conducted.

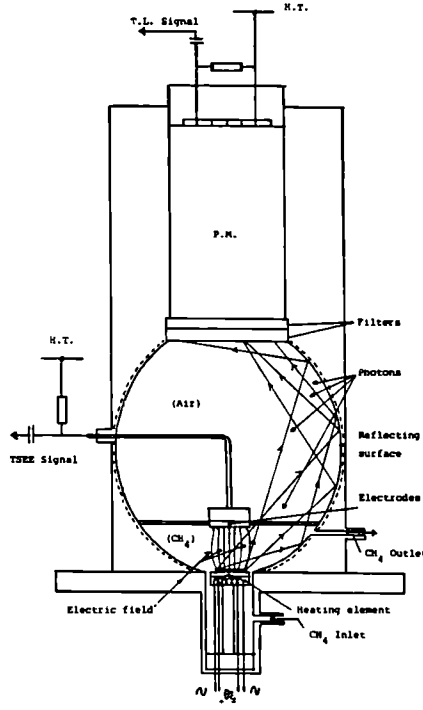


Figure 1 : EETS and TL detection system

IV. Objectives for the next reporting period:

Use of a mixed head together with BeO film/BeO ceramic dosemeters ; tests on fast TSEE/TL reader for routine measurements.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)] :

Professor A. SCHARMANN
I Physikalische Institut des Universitat Giessen
Heinrich, Buff-Ring 16
6300 GIESSEN - FRG -

VI. Publications:

W. KRIEGSEIS, M. PETEL and A. SCHARMANN
"Response of BeO thin film dosemeters to beta irradiation".
9th International Symposium on Exoelectron Emission and Applications.
WROCLAW Pologne, October 3-8 (1988)

M. PETEL, J. BARTHE and G. PORTAL
"A new computerized TSEE Reader"
9th International Symposium on Exoelectron Emission and Applications.
WROCLAW Pologne, October 3-6 (1988)

Title of the project no.: 2

Thermoluminescence and Electron emission from cotton fabrics.

Head(s) of project:

J. BARTHE

Scientific staff:

C. HICKMAN - Ph. BLANCHARD

I. Objectives of the project:

The method is based on the use of a single dosemeter which can be read either simultaneously or consecutively by radiothermoluminescence (RTL) and thermally stimulated exoelectron emission (TSEE). The difference between the responses obtained using these two techniques arises from differences between optical transmission through a depth of a few hundred micrometers in the case of RTL and electron transmission from a depth of between 1 and 10 nm from the detector surface in the case of TSEE.

II. Objectives for the reporting period:

The main objectives for this reporting period relating to studies of fabrics loaded with different quantities of alumina of various grain sizes are presented. Two kinds of difficulty have been encountered depending on the readout systems used, exoelectron emission or thermoluminescence.

Much of the work involved investigating thermal and optical fading because clothes are also worn out doors.

III. Progress achieved:

1. Introduction

In the case of an accident, the dosimetric information must be obtained in a very short lapse of time. To perform dose measurements four methods has been envisaged : two of the physical methods suggested : thermo-stimulated exoemission and phototransfer induced thermoluminescence may be performed directly on small fabric samples. Alterations in the TL signal occur during heating due to burning of the cellulose. The remaining two methods relate to the thermolumines cence of fabric samples and measurements on alumina after extaction by chemical means.

The results obtained in these different cases depend on several parameters e g. grain size, density, specific and free surface areas. The last method takes 10 hours to perform.

2. Results

Grain size is selected by making a compromise between dosimetric characteristics and fabric textile effects. For this reason, the maximum grain size used in these experiments is less than 40 μm.

- Thermostimulated exo-electron emission

In the case of exo-electron emission, the coloured appearance due to the burning of the fabric has no effect on electron transmission from the inside to the outside of the alumina grains. For this reason exo-emission is the simplest method for dose determinations. In these experiments, a multineedle counter with a focasing anode has been used. The electrical charge effects encountered with heated fabric samples do not change the dosimetric response. The intrinsic exo-emission of cotton fabric is lower than the alumina response.

Figure 1 shows the TSEE response of alumina loaded cotton irradiated with X, γ and β rays. The dose deposited is about 200 mGy.

Measurements reproducibility is better than 10%. In the case of TSEE, a post calibration may be used to determine the correct sensitivity.

Figure 2 shows the response curve in terms of integrated counts versus air kerma dose for fabrics loaded with different surface densities.

- Thermoluminescence

Because of fading effects observed with low temperature photostimulated peaks, the high temperature peak close to 250°C was used for measurements.

Two methods can be used to determine the fabric's sensitivity to radiations

- Additive method
- Use of TSEE calibrations related to TL measurement from well known fabric responses.

In contrast with the first method, the second method can be used to determine sensitivity and background signal of the fabric used.

Figure 3 shows the TL glow curve of alumina doped cotton. The intrinsic TL of the cotton is very low and combustion light effects may be neglected if readout is performed using nitrogen or another inactive gas for flushing.

Measurement reproducibility is within the same order of magnitude as TSEE ± 10% for doses of about 200 mGy.

Figure 4 shows the TL response of alumina loaded cotton fabrics irradiated with γ-rays from Cobalt-60.

Conclusion

The main difficulties encountered with the use of alumina loaded cotton fabric now seem to have been solved :

- **preparation of alumina loaded cotton fabbrics**
- **readout procedures.**

Some difficulties due to domestic usage still have to be solved such as : response stability after a series of washings (with or without chloride products in the water).

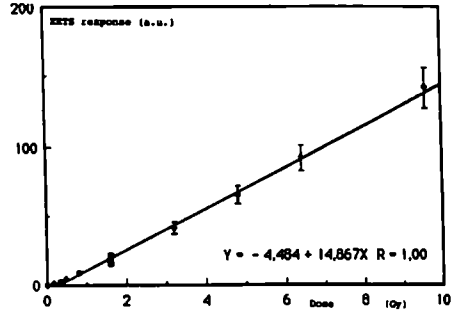


Figure 1 : EETS response versus Sr-90 irradiation dose

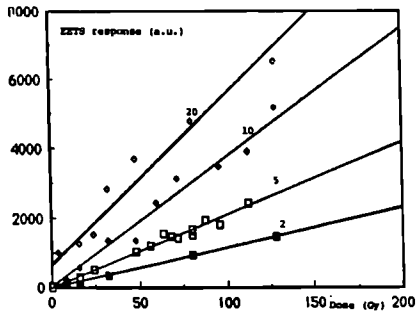


Figure 2 : EETS response versus irradiation dose for 4 surface densities of Alumina

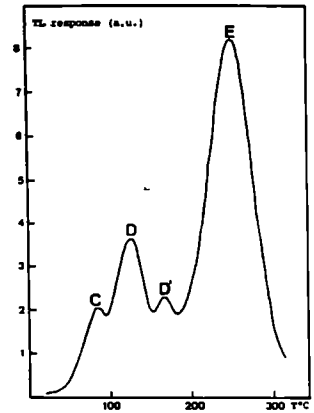


Figure 3 : Glow curve of Alumina deposited on cotton

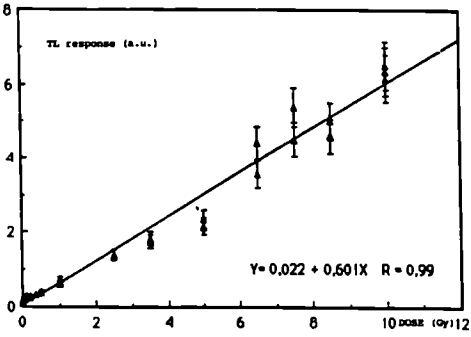


Figure 4 : TL response versus Sr-90 irradiation dose

IV. Objectives for the next reporting period:

The main objective for 1989 is the development of a complete dosimetric system including fabric manufacture, reading procedures and the simulation of gamma irradiation accident cases enabling overall efficiency to be determined under real conditions.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)] :

Laboratoire d'Emission Electronique et de luminescence Université de Nice, Parc Valrose, 06000 Nice - F -
Laboratoire de physico-chimie des matériaux
Ecole Nationale des Mines de St Etienne - St Etienne - F -

VI. Publications:

D. LAPRAZ , P. IACCONI, Y. SAYADI, P. KELLER, J. BARTHE and G. PORTAL "Some thermoluminescence properties of an α-Al_2O_3 sample. Sensitization effects".
Physica Status Solide a, 108, (1988) pp 783-794.

Title of the project no: 3

Dosimetry on natural and synthetic fabrics by ESR.

Head(s) of project:

F. BERMANN - J. BARTHE

Scientific staff:

R. CHUITON - J.M. BORDY

I. Objectives of the project:

The method is based on the use of a single dosemeter which can be read either simultaneously or consecutively by radiothermoluminescence (RTL) and thermally stimulated exoelectron emission (TSEE). The difference between the responses obtained using these two techniques arises from differences between optical transmission through a depth of a few hundred micrometers in the case of RTL and electron transmission from a depth of between 1 and 10 nm from the detector surface in the case of TSEE.

II. Objectives for the reporting period:

The main objectives of the work reported during this period relate to the predose or background effects exhibited by a non irradiated fabric and evaluated in terms of dose.

Studies have been orientated in two directions : investigations into the effects of physical and chemical treatments applied to fabrics before measurements ; evaluation of the initial background signal from the fabric using mathematical and numerical methods.

The problems are more complex than originally supposed. A lot of difficulties have been encountered : the flatness of the cotton base line being one of the most preponderant problems.

III. Progress achieved:

In previous reports, some of the thermal characteristics due to the effects of environmental parameters on cotton and polypropylene fabrics e.g. thermal fading have been described ; humidity and sun light have also been observed to affect the signal.

Some progress has been achieved in lowering the background signal from cotton. In addition to physical and chemical treatments such as dehydration under vacuum at room or high temperatures, it has been observed that heatings with durations ranging from a few seconds up to a few minutes can slightly decrease the background signal due to the hydroxyl radicals being retained by the fibers.

These observations complete studies on the thermal fading occurring with cotton and polypropylene fabrics. Care was taken to avoid secondary effects such as mechanical and electrical frictions.

Figure 1 and 2 show the thermal fading exhibited at room temperature by cotton and polypropylene respectively. A rapid decay is observed less than 10 hours after irradiation with cotton ; this is followed by a stable signal particularly for relatively low doses (5Gy). The decay in the signal from polyethylene is quite comparable to cotton.

The main effects due to thermal activation applied during a short period of time are as follows :

- water elimination leading to an increase in the ESR signal due to a lower microwave power absorption,
- thermal fading leading to a more rapid stabilization (less than one hour).

Figure 3 shows the activation coefficient versus temperature for cotton and polypropylene fabrics. The values evaluated are : 0.60 eV and 2.24 eV for cotton and polypropylene respectively.

In order to evaluate doses from cotton samples in the absence of any information on background signal, the cumulative dose method is applied to the same fabric sample.

It is assumed that signal/dose curves are the same for all batches of cotton the reference curve can then be superimposed on the unknown curves with appropriate horizontal and vertical translations. The real dose value can then be deduced from the translation values.

Figure 4 shows the reference curve used for such determinations.

The characteristics presented indicate the limiting values that can be determined today with the present state-of-the-art ; pre-existing free radicals occur in the fiber structure and cannot be avoided.

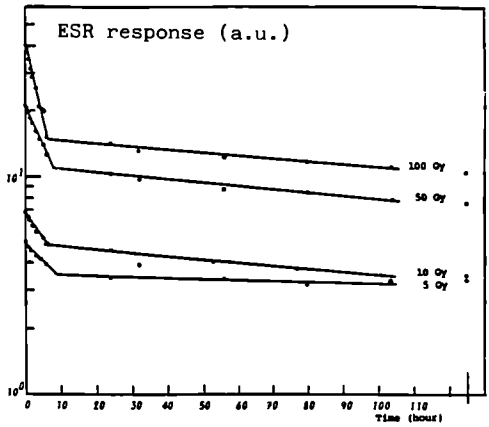
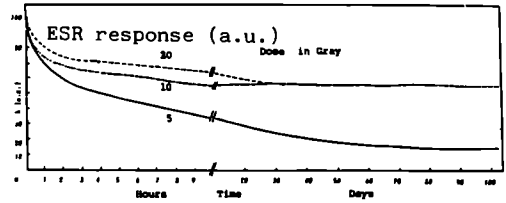


Fig. 1 : Fading of natural cotton at different doses



Fig; 2 : Fading of synthetic polypropylene fabrics at different doses

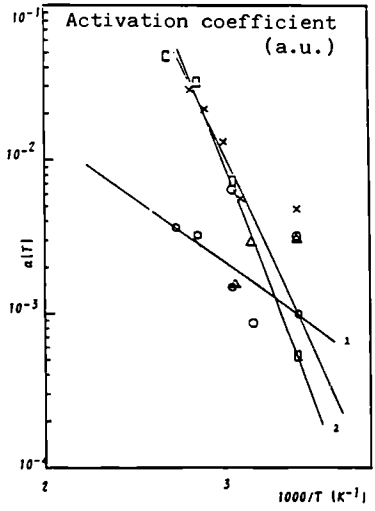


Fig. 3 : Activation coefficient versus 1000/T. Slopes are proportional to activation energy : 1/Cotton, 2/ Polypropylene

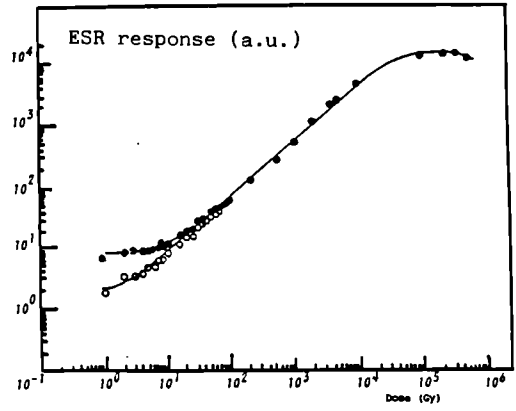


Fig. 4 : ESR reference curve used with cotton : background ● included
o subtracted

IV. Objectives for the next reporting period:

Measurement procedures have now been well established for cotton and polypropylene. The next step will be to use the techniques developed to simulate real cases, to improve preparation procedures and to apply appropriate physical and chemical treatments. Measurement procedures will be optimized.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)] :

Institut Textile de France
BP n°60
69132 ECULLY Cedex - France

VI. Publications:

V. KAMENOPOULOU
"Proprietés dosimétriques des fibres textiles"
Rapport CEA-R-5425 (1988)

J. BARTHE, V. KAMENOPOULOU, B. CATOIRE and G. PORTAL
"Dose evaluation from textiles fibres. A post determination of initial ESR signal"
2nd Symp. on ESR Dosimetry and Applications
Munich/Neuherberg, October 10-13 (1988)

Title of the project no.: 4

Realization of an "operational" spectrometry unit for neutrons.

Head(s) of project:

J.L. CHARTIER

Scientific staff:

F. POSNY - R. MEDIONI - J. POITREAU

I. Objectives of the project:

The "operational" spectrometry unit should be able to ensure the spectrometry of the radiation fields encountered in practice for radiation protection purposes. For such measurements, the spectrometric system must have some essential qualities (good energy resolution, quasi-isotropic angular response. discrimination between neutrons and photons...) which have been approachec as close as possible by the use of two spherical gas-filled proportionnal counters (type SP2-Winfrith-UK) and one liquid scintillator probe (type NE213).

II. Objectives for the reporting

For 1988 the main objectives were :

- to measure or confirm some characteristics of the detectors with monoenergetic neutron beams (Van de Graaf accelerator at the CEN Bruyères-le-Châtel)
- to take into account these experimental data in the unfolding codes and check the symplifying hypotheses in the calculations
- to work out the wide spectra measurements done at the CEN Cadarache.

III. Progress achieved:

1. Gas-filled spherical proportionnal counters $\begin{cases} n°1 \cdot H_2\ 3bars \\ n°2 \cdot CH_4\ 5bars \end{cases}$

Measurements with monoenergetic neutrons were carried out between 60keV and 2MeV in January and April 1988. The values deduced from all the measurements (1987-1988), viz response functions shape factors and efficiency curves, have been included into the unfolding code to take better into account the experimental characteristics of the detectors.

The main symplifying hypotheses have also been tested and found sufficiently realistic not to be modified.

2. NE213 probe

The efficiency variation with the neutron energy has been measured between 1MeV and 2.6MeV, and at 14.7MeV (BIPM 1987). This curve was compared with calculated values (O5S code) and taken into account in unfolding codes using the differentiation method or a similar one.

The parasitic contributions due to the secondary reactions on the carbon nuclei and to the high energy neutrons have been estimated for 14.7MeV by an experimental mean and deduced, when it is necessary, of the measured spectra.

3. Wide spectra spectrometry

New measurements with the NE213 probe (February 1988) at the CEN Cadarache have allowed to reconsider the results of 1987 with the modified unfolding codes.

Two radiation fields have been studied :

- a "fission" spectrum : interaction of 14.7MeV monoenergetic neutrons with a 12 cm thick uranium 238 shell
- a wide spectrum obtained by addition to the previous set-up of a 15 cm thick iron shell.

The energy range has been analysed as follows : from 20keV to 500keV by the spherical counter filled with hydrogen (3 bars), from 400keV to 1.7MeV by the spherical counter filled with methane (5 bars), and from 1.5MeV to 17.5MeV by the NE213 probe. The results are shown in figures 1 and 2.

$K_{reference}$: 5,33
$K_{calculated}$: 5,90
} 10^{-9} Gray per monitor count

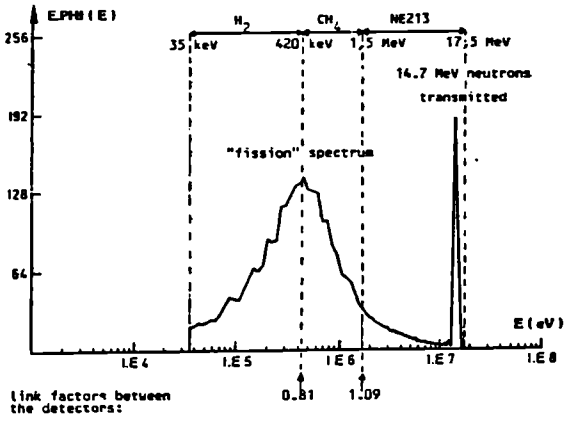


Figure 1.- CONFIGURATION N°1: 14.7 MeV + ^{238}U

$K_{reference}$: 1,76
$K_{calculated}$: 2,14
} 10^{-9} Gray per monitor count

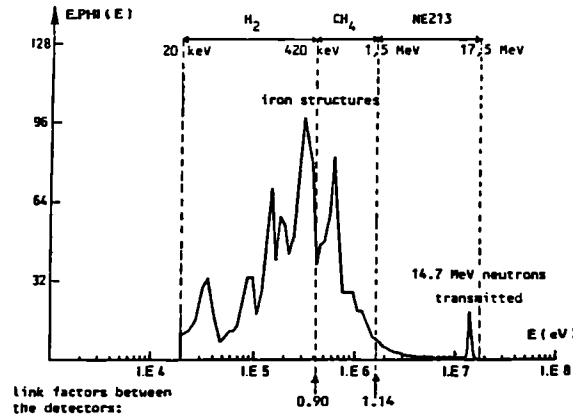


Figure 2.- CONFIGURATION N°2: 14.7 MeV + ^{238}U + Fe

The reference value chosen to check the method validity is the neutron kerma value measured with the twin-detector technique. The corresponding value calculated from the measured spectra overestimates from 10 and 20% the reference value. This discrepancy could be attribute to parasitic effects due to the interaction of high energy neutrons with the spherical counters and to carbon recoils in the methane filled counter. These contributions have to be estimated in 1989.

IV. Objectives for the next reporting period:

- Study of the parasitic effects due to high energy neutrons for the two spherical counters.
- Estimation of the carbon recoil correction for the methane filled spherical counter.
- Measurement of new wide spectra.
- Definition of the dose equivalent sensitivity limits of the "operational" spectrometry unit and evaluation of the uncertainties.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)] :

In July 1988, discussions on results with a laboratory of the P.T.B. implied in the same field and using the same techniques have led to a program of intercomparison on the wide spectra of the CEN Cadarache facility.
Dr Klein-Dr Knsuf - Neutron group - PTB Braunschweig (RFA).

VI. Publications:

A poster presenting the spectrometric system has been showed at the 7th International Conference on Radiation Shielding in Bournemouth (U.K) in September 1988.

A paper will be published in the Proceedings of the Conference.

Title of the project no: 5

Study and realization of an individual dosemeter based on photographic emulsion.

Head(s) of project:

G. PORTAL

Scientific staff:

Ph. BLANCHARD - C. HICKMAN (CRN-Strasbourg)

I. Objectives of the project:

Study and realization of an individual neutron dosemeter based on photographic emulsion.

Replacement of the microscopic track counting method for nuclear emulsions by an activation method to determine the amount of silver present in an emulsion as a result of (n,p) reactions...

II. Objectives for the reporting period:

- Application of X-ray fluorescence detection to determine the amount of silver ; determination of the minimum detectable dose.

- Reduction of background to lower detection threshold when the activation method is used.

III. Progress achieved:

Application of X-ray fluorescence

The objective was to determine the neutron detection limit when the amount of remaining silver is measured by a fluorescence method and to compare the results with the activation method.

Two X-ray fluorescence apparatus have been used :

- a commercially available device...
- an X-ray fluorescence system constructed in the lab for this purpose.

The results show this method to be insensitive with either type of measuring device. The lower detection limit is about 5 mGy, higher than the detection limit obtained with the activation method : 1 mGy.

Reduction of background when the activation method is used

The detection threshold has been reduced to 0.5 mGy using a substraction method. An inconvenience of this technique is its inability to detect neutrons in the range of energy : 100-600 keV.

Conclusion

The limits of applicability of photographic emulsions to neutron individual monitoring by the activation method or X-ray fluorescence method have been investigated. It is considered that no further progress can be made. It is proposed to end research in this field.

IV. Objectives for the next reporting period:

The study will not continue.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]

S.A.D.V.I Centre de Recherches Nucléaires de Cronenbourg Strasbourg

VI. Publications:

C. HEILMANN - Thesis N°207 "Dosimetrie des rayonnements par activation neutronique ou fluorescence X de l'argent résiduel dans les émulsions photographiques". Centre de Recherches Nucléaires de Cronenbourg. Université Louis Pasteur Strasbourg.

Title of the project no.: 6

Aera and Individual Dosimetry with proportinal counters

Head(s) of project:

J. BARTHE - M. PETEL

Scientific staff:

R. CHUITON - J.C. CHAPUIS

I. Objectives of the project:

The method is based on the use of a single dosemeter which can be read either simultaneously or consecutively by radiothermoluminescence (RTL) and thermally stimulated exoelectron emission (TSEE). The difference between the responses obtained using these two techniques arises from differences between optical transmission through a depth of a few hundred micrometers in the case of RTL and electron transmission from a depth of between 1 and 10 nm from the detector surface in the case of TSEE.

II. Objectives for the reporting period:

The main work accomplished during rhe reporting period has essentially been related to the correlation between experimental results and numerical calculations of gaseous gain based on Bolzmann's equation for thermodynamic equilibrium. The difference between experimental and calculated values has led to some new assumptions being made.

III. Progress achieved:

All the numerical calculations have been made for the case of cylindrical geometry : the range of values considered is comparable to that covered bby experimental counters.

The distribution functions along the axis of symmetry are assumed to be constant and lead to cumulative effects.

The first figure shows the logarithm of the gain versus applied voltage. It is readily seen that the plateau does not occur at the beginning ; the ionization chamber regime and proportional regime are concomitant ; the magnitude of non amplified pulses is obtained by extrapolating to high applied voltages and from the difference between total and estimated amplified gain magnitudes.

Figures 2 and 3 show the gaseous gain for Argon and CH_4. The solid line corresponds to calculated values ; the dotted line corresponds to the curve enveloping the experimental points. Several different reasons explain the difference between these two lines.

1. Experimental reasons

- incorrect evaluation of the electron source term due to concomitant effects such as gaseous gain in the high electric field region near the anode wire and recombination in the low electric field region near the anode,

- incorrect reference evaluation of the electron source term due to non-existence of the collecting plateau,

- shape modification of collected current pulses.

2. Calculation reasons

- non validity of thermodynamic equilibrium in the case of high electric field gradients,

- incorrect estimation of the first ionization coefficient.

Figure 4 shows the correlation between experimental and calculated gain values versus an indentical applied high voltage. A shift in the concurrent experimental and calculated curves towards higher pressures can be observed. This is correlated with the underestimation of the apparent first ionization coefficient due to curving effects experienced by the electrons generated around the anode. This effect increases with decreasing pressure and applied voltage.

Conclusions

The above described results are more complex than we initially envisaged. Some special efects have not yet been described.

Some of the assumptions made appear to be valid e.g. electric field gradient can be reduced by using a large anode wire diameter ; non equilibrium effects may be due to wall effects.

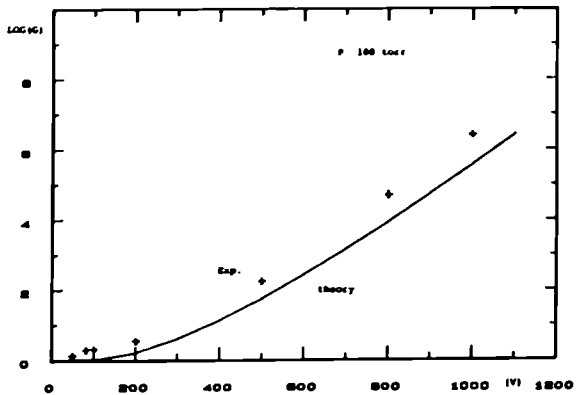


Fig. 1 : Gas gain versus applied voltage for CH_4 - Ar (20%)

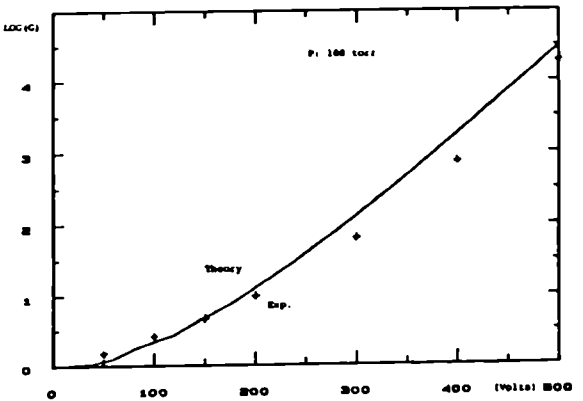


Fig. 2 : Gas gain versus applied voltage for Argon

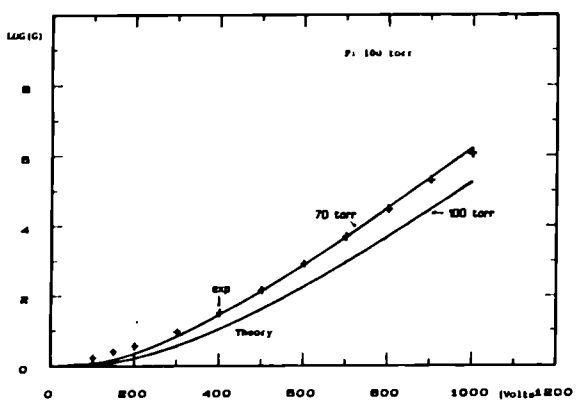


Fig. 3 : Gas gain versus applied voltage for CH_4

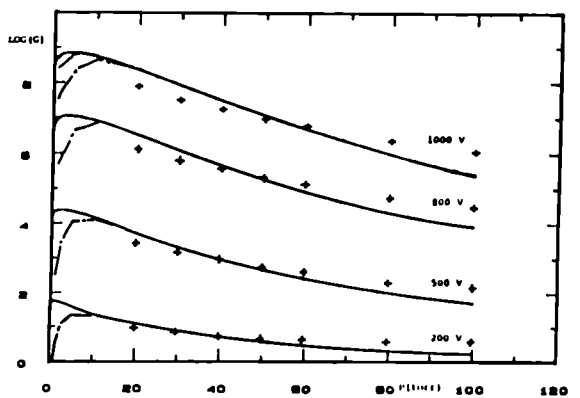


Fig. 4 : Gas gain versus CH_4 pressure

IV. Objectives for the next reporting period:

Up to now, most tissue equivalent proportional counters have been designed with an anode wire with or without a helix. When a helix is employed some undesirable effects such as microphonic effects during handling and walking must be eliminated ; this is specially true for individual monitoring if low detection thresholds are to be obtained. It is thus envisaged to replace the anode wire with a set of focusing field anode structures similar to those used in the multineedle exoelectron emission counter.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)] :

Groupe de recherche sur les décharges à faible intensité
Centre de Physique Atomique, Université P. Sabatier
31062 TOULOUSE Cedex - France -

VI. Publications:

P. SEGUR, I. PERES, J.P. BOEUF and M.C. BORDAGE
Microscopic calculation of the gaseous gain in cylindrical proportional counters
Workshop on inplementation of tissue equivalent proportional counters in microdosimetry
19-21 October 1988, Scloss Elmau, RFA
to be published in Rad. Prot. Dos.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université Louis Pasteur
11, rue Humann
F-67085 Strasbourg Cédex

Contract no.: BI6-A-021-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. R.V. Rechenmann
LBRM - INSERM U.220
Université Louis Pasteur
11, rue Humann
F-67085 Strasbourg Cédex

Telephone number: 88-35.13.27

Title of the research contract:

Heavy charged particle track structure in tissuelike media, incidence on microdosimetric interpretations.

List of projects:

1. Heavy charged particle track structure in tissuelike media, incidence on microdosimetric interpretations.

Title of the project no.: BI6 - A - 021 - F

Heavy charged particle track structure in tissuelike media, incidence on microdosimetric interpretations

Head(s) of project: R.V. RECHENMANN

Scientific staff: R.V. RECHENMANN
B. SENGER
E. WITTENDORP-RECHENMANN

I. Objectives of the project:

The contracting Laboratory is intended to continue the track feature measurements along heavy charged particle (hcp) trajectories materialized in ionographic detectors. These data have to be interpreted in terms of hcp interactions with dense matter. In a second stage, the codified fundamental interaction processes will be used for building up a realistic ionizing track pattern which will be later on introduced in interpretative radiobiological action models.

II. Objectives for the reporting period:

The experimental investigations related to the quantitative evaluations of heavy charged particle track parameters materialized in ionographic media have been pursued, notably the measurements concernig the intergranular gap distributions along α-particle tracks. The intercomparison by means of computer-assisted graphical representations of double-differential cross-sections (DDCS) obtained by various theoretical approaches for the ionisation of substances of radiobiological interest by hcp's has been continued. The development of semi-empirical formulas of the elastic scattering cross-section, differential in respect to the electrons's scattering angle, or integrated, has been initiated. Furthermore, the extension to primary electrons of the DDCS-MT, developed for the description of the ionisation of atoms and molecules induced by heavy charged particles (hcp), has been undertaken. Both sets of expressions will contribute to the edification of a modular numerical track structure simulation code.

III. Progress achieved:

Intergranular gap distributions

The investigations concerning the quantitative evaluation of particle track parameters in tissuelike dense targets have been pursued by using the ionographic detection system developed in the Laboratory. Very preliminary track feature measurements seemed to indicate that "gaps", distributed along proton or α-particle tracks recorded in highly sensitive nuclear emulsions, tend to concentrate at defined locations along the trajectories (see previous reports). In order to decide whether this feature can be effectively associated with a reproductible phenomenon, and eventually related with energy loss mechanisms, e.g. the ionisation process, of heavy charged particles, the systematic study on this parameter has been continued. A specific interactive image analysis procedure had to be worked out for making precise and reproducible measurements on intergranular gap distributions with lengths λ ($\lambda \geq 0.08$ μm). A series of preliminary test measurements had also to be undertaken in order to establish a suitable analysis protocol, notably by rigorous counting conventions and by verifying the repeatibility performances for various experienced operators, etc. Specific treatment softwares have been designed for the evaluation of the experimental data. Measurements on α-particle tracks are actually underway.

Comparison of ionisation theories by means of four-dimensional graphical representations

Ionisation double-differential cross-sections (DDCS) have been calculated by means of the DDCS-MT (see previous reports) and the DDCS-BEA from the literature, in the case of methane traversed by protons of energy E = 0-10 MeV. An efficient tool for comparing both series of data is provided by the computer-assisted **graphical representation** method, already described in the 1987 report and applied to single-differential cross-sections. Figure 1 represents the product T × DDCS as a function of E, and of the ejection energy (T) and angle (θ) of the δ-rays. The fourth dimension is given by the grey level directly correlated with T × DDCS. This drawing reveals clearly the large differences between both approaches, especially at the large ejection angles and small ejection energies. Such illustrations are particularly powerful for **revealing differences**, sometimes difficult to apprehend by other means, between two or more approaches intended to describe a given mechanism.

Elastic scattering of slow electrons

A study has been undertaken on the **elastic scattering of slow electrons**, which influences the position of the energy depositions around an incoming heavy charged particle. Since the relative "weight" of this mechanism, if compared to ionisation or excitation, increases with decreasing energy, emphasis is placed on the energy domain 0-200 eV, where the experimental differential cross-sections (DCS) deviate strongly from the predictions of the screened Rutherford formula. Preliminary comparisons with measurements related to low-Z molecules available in the literature, showed that it may be possible to represent the DCS by **parametric analytical functions**. As to the integrated cross-sections, an analoguous procedure has been adopted.

Ionisation by medium energy electrons

Theoretical investigations on a possible **extension of the DDCS-MT to primary electrons** have been initiated by modifying the mathematical expressions constituting this approach in order to remove the approximations valid only in the case of hcp's. Tentative exchange factors will also be introduced in the resulting expression in order to account for the identity of the two emerging electrons. In a first stage, only primary electrons which are not too slow and not to high energy transfers will be considered, in order to justify the representation of the scattered electrons by plane waves.

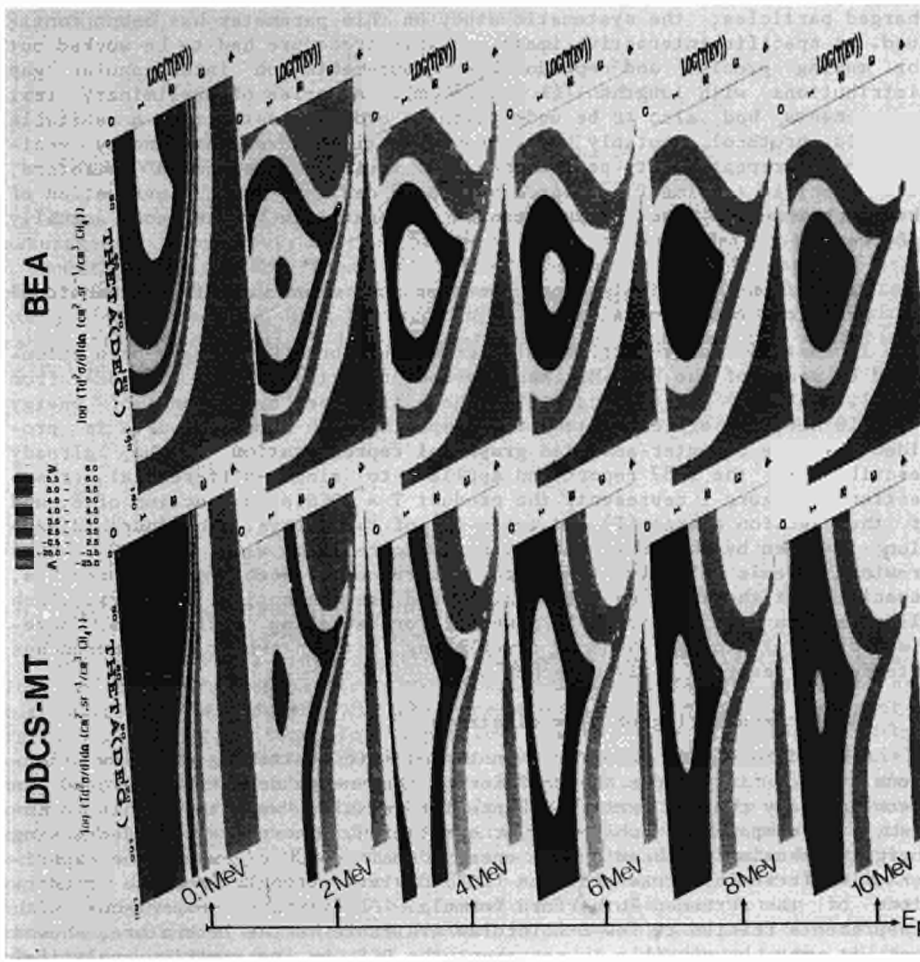


Fig.1

IV. Objectives for the next reporting period:

The track analysis concerning the distribution of intergranular gaps along heavy charged particle trajectories materialized in nuclear emulsions will be pursued. The obtained frequency distributions will be submitted to a detailed statistical analysis as well as to considerations concerning the geometrical configuration of the ionographic detection system. An elucidation of these observations by means of calculations, eventually based on ionisation cross-sections, will be undertaken.

The studies aimed at the development of parametric formulas describing the cross-section of the elastic scattering of low-energy electrons will be continued in order to determine their applicability domain. The possibility to extend the DDCS-MT to the ionisation of atoms, and especially molecules of biological interest, by primary electrons, will be submitted to further investigations.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- FOM-Institute for Atomic and Molecular Physics, Amsterdam (The Netherlands) (Dr. J.B. SANDERS).
- Laboratoire "Physique Moléculaire et Collisions", C.M.S.R., Université de Metz (Pr. Cl. TAVARD).
- Unité n° 311 de l'I.N.S.E.R.M., Centre Régional de Transfusion Sanguine, Strasbourg (Pr. J.-P. CAZENAVE).

VI. Publications:

- B. SENGER, J.-L. VONESCH and R.V. RECHENMANN. **Application of computer-assisted multi-dimensional graphics in microdosimetry : ionisation of methane by 0-10 MeV protons.** Radiat. Prot. Dosim. 23 (1988) 53-56.
- E. WITTENDORP-RECHENMANN, J.-L. VONESCH, R.V. RECHENMANN, C. KLEIN-SOYER and J.-P. CAZENAVE. **Development of a computer-assisted methodology combined with a specific autoradiographic method. Application to the study of human endothelial cell regeneration.** Innov. Tech. Biol. Med. 9 (1988) 17-28.
- B. SENGER. **Calculated molecular double-differential cross-sections for ionisation under proton impact.** Atoms, Molecules and Clusters (Z. Phys. D) 9 (1988) 79-89.
- E. WITTENDORP-RECHENMANN, J.-L. VONESCH, V. KOZIEL-VIGNERON and R.V. RECHENMANN. **Modular interactive opto-electronic system for track structure analysis.** Radiat. Prot. Dosim. 23 (1988) 199-202.

Communications, Proceedings and Abstracts

- S. FALK, B. SENGER et R.V. RECHENMANN. **Diffusion des électrons produits au cours de l'ionisation de molécules par des particules chargées lourdes.** Actes des journées d'études (Université de Paris-Sud) : "Collisions (e,2e) et problèmes connexes" ; ed. Cl. Tavard (Université de Metz, 1988) 23-28.

- S. FALK, B. SENGER et R.V. RECHENMANN. **Diffusion élastique d'électrons de faible énergie par des molécules d'intérêt radiobiologique.** Actes du 12ème Colloque sur la Physique des Collisions Atomiques et Electroniques (Caen, 1988) Vol. I, 83-84.

- C. KLEIN-SOYER, A. BERETZ, J.-P. CAZENAVE, E. WITTENDORP-RECHENMANN, J.-L. VONESCH, R.V. RECHENMANN, F. DRIOT and J.-P. MAFFRAND. **Repair process of a mechanical lesion of irradiated endothelial cells : Modulation by standard heparin alone or in association with acidic fibroblast growth factor (aFGF).** VIth International Symposium on the Biology of the Vascular Endothelial Cell (Toronto, 1988) (Abstract).

- B. SENGER, E. WITTENDORP-RECHENMANN and R.V. RECHENMANN. **Ionisation cross-sections for heavy charged particles traversing tissuelike media.** Proc. of "EULIMA Workshop on the potential value of light ion beam therapy" (Nice, 1988) (in press).

- Y. PETEGNIEF, B. SENGER et R.V. RECHENMANN. **Formalisme de sections efficaces différentielles d'ionisation des molécules H_2 et H_2O par des électrons de 100 à 1000 eV.** Actes des journées d'études (Université de Paris-Sud, 1988) : "Collisions (e,2e) et problèmes connexes" ; ed. Cl. Tavard (Université de Metz) (in press).

Doctorat (Mention Sciences) de l'Université Louis Pasteur de Strasbourg I (1988).

- S. FALK, **"Diffusion élastique d'électrons (1 eV-200 eV) par des molécules d'intérêt radiobiologique - Contribution à la modélisation de la structure des traces de particules chargées".**

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Kernforschungszentrum Karlsruhe
KFZ
Postfach 3640
D-7500 Karlsruhe

Contract no.: BI6-A-029-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. D. Taylor
Inst.f.Genetik und Toxikologie
v. Spaltstoffen - KFZ Karlsruhe
Postfach 3640
D-7500 Karlsruhe

Dr. E. Polig
Inst.f.Genetik und Toxikologie
v. Spaltstoffen - KFZ Karlsruhe
Postfach 3640
D-7500 Karlsruhe

Telephone number: 7247-82.32.91

Title of the research contract:

Microdosimetry and local dosimetry of 226-Radium, 239-Plutonium and 241-Americium in the beagle dog skeleton.

List of projects:

1. Microdosimetry and local dosimetry of 226-Radium, 239-Plutonium and 241-Americium in the beagle dog skeleton.

Title of the project no.: BI-6-0029-D

Microdosimetry and local dosimetry of Ra-226, Pu-239 and Am-241 in the beagle dog skeleton.

Head(s) of project: Prof. Dr. D. M. Taylor, Dr. E. Polig

Scientific staff: Dr. E. Polig
Prof. W.S.S. Jee
(University of Utah/U.S.A.)

I. Objectives of the project:

To determine the microdistribution of the radiation dose from internally deposited Ra-226, Pu-239 and Am-241 in selected bones taken from beagle dogs in the University of Utah life-span studies.

To improve understanding of the mechanisms of bone tumor induction in dogs and man by alpha-emitting radionuclides from the nuclear fuel cycle in order to provide better risk estimate models for man.

II. Objectives for the reporting period:

Continuation of the analysis of autoradiographs from bones of beagle dogs contaminated with Ra-226.

Design of a relative risk model for Pu-239 induced tumors for the major parts of the beagle skeleton, based on morphometric and histomorphometric parameters.

Design of a model for the closure of osteonal cavities useful for calculating radiation doses to osteoblasts during the period of osteon formation.

III. Progress achieved:

1. Methodology

The microdistribution and local concentrations of Ra-226 in trabecular sites of the beagle skeleton were determined by exposing CR39 autoradiographic track detectors on contaminated bone sections. After exposure bone sections were stained with Alizarin-red to display calcified tissue. CR39 detectors were etched in NaOH to develop latent alpha-tracks into microscopically visible etch pits. Both the stained bone sections and the radiation detector were then scanned using a computer-controlled scanning microscope photometer. This measurement technique provided a digitized image of the trabecular structure in the area scanned and the associated alpha-track distribution from Ra-226 in a sampling grid of 20 µm unit length. The microscope photometer was calibrated by means of standard sources uniformly labeled with known concentrations of Ra-226, and density and chemical composition similar to bone. The fractional Rn-222 retention in embedded bone sections was 32%, the detection efficiency of Ra-226 was 0.25 and of the alpha-emitting daughters 0.15. Until now results were obtained from lumbar vertebra bodies and proximal ulnae of young adult beagles receiving a single injection of 370 or 37 kBq/kg Ra-226.
The calculation of dose factors at plane bone surfaces and in cortical osteons was carried out by means of a Monte-Carlo simulation of alpha-particle decays in bone volume or at bone surfaces.

2. Results

A distinctly faster release of Ra-226 was observed from the lumbar vertebra than from the proximal ulna. At early times after injection, and in animals with 37 kBq/kg administered dose, the average concentration of Ra-226 was about 2000 Bq/g-bone in the l. vertebra and 400 Bq/g-bone in the prox. ulna. At 1000 days both concentrations were equal to 200 Bq/g. About 30% of the total activity was found in Ra-226 hotspots. The concentration ratio of hotspot/diffuse labels varied between 8-10. The number of hotspots/mm^2 was initially lower in the prox. ulna (0.5/mm^2) than in the l. vertebra (1.5/mm^2) but with

Table 1 Summary of dosimetry in lumbar vertebra and proximal ulna[a]

	Concentration Bq/g-bone	Max dose rate mGy/day	Max. hit rate hits/day	Total hits[b]
		Bone lining cells at diffuse labels		
L vertebra	950	30	0 038	7
Prox ulna	300	9 5	0 012	7
		Osteoblasts at expanding hotspots		
L. vertebra	6500	160	0 21	10
Prox ulna	2800	70	0 09	4

[a] 37 kBq/kg body weight ^{226}Ra as single injection.

[b] assuming 180 days and 600 days residence time for lining cells in the l vertebra and prox ulna, and a bone formation period of 50 days for osteoblasts

increasing post injection times approached the common value of 1/mm^2. Hotspot areas were in the range 4000-8000 µm^2. Table 1 lists some of the dosimetric results for early post injection times.

Soon after injection nuclei of lining cells receive a dose rate of 30 or 9.5 mGy/day in l. vertebrae or prox. ulnae, respectively, at diffuse deposits of Ra-226. This corresponds to about 7 hits to the cell nucleus per cell generation in both types of bone. Calculated dose rates in the l. vertebrae were declining, but were practically constant in the prox. ulna over the period of observation (4-3000 days) (Fig. 1).

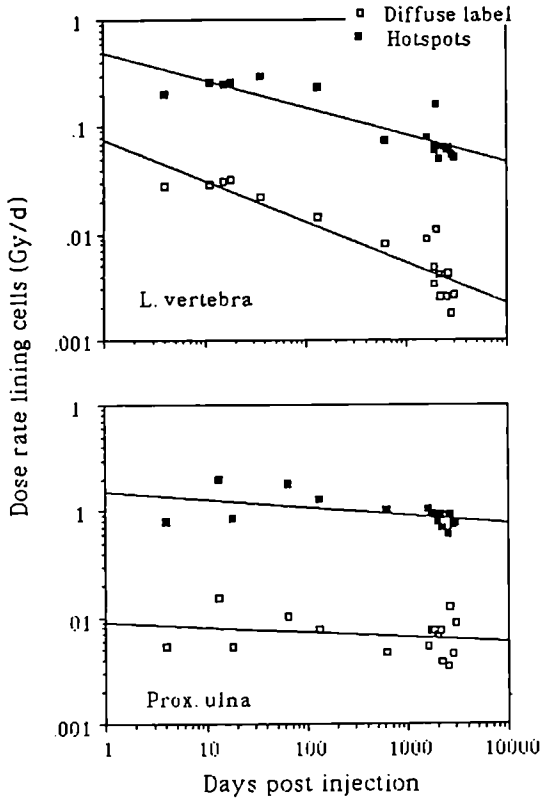


Fig. 1 Radiation dose rate to bone lining cells in trabecular portions of the lumbar vertebra and proximal ulna of beagles after a single injection of 37 kBq/kg Ra-226.

In animals with an administered dose of 370 kBq/kg, an imbalance between bone resorption and formation was observed, resulting in a net increase of bone mass and trabecular thickness. Such an imbalance was absent in the group with 37 kBq/kg Ra-226.

To describe the distribution pattern of bone surface-seeking actinides such as Pu-239 and Am-241, a kinetic model of uptake, deposition and redistribution has been designed. The model is based on the concept of flow of the nuclide in blood and attempts to predict the dynamic change of the labelling pattern as a result of release and recirculation of the nuclide from its primary sites of deposition. It consists of a liver compartment, two skeletal compartments representing cancellous and compact bone, respectively, and a single excretion pathway (Fig. 2).

The interior dynamics of labels in the skeletal compartments was described mathematically using the concepts of quantal bone remodeling within bone structural units. Using, as an example, retention data of Pu-239 in young adult beagles after a single injection, it was found that initially 4.1% of the injected activity was in cortical and 46% in cancellous bone. The clearance rates from both compartments were in

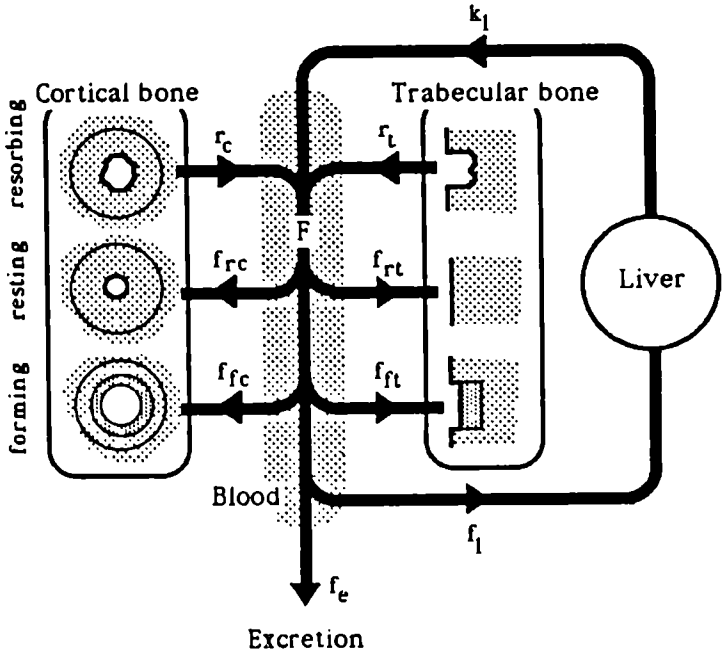


Fig. 2 Kinetic model of plutonium distribution and excretion in the main deposition organs after injection into blood. F = total flow of Pu released from liver and bone, k_l, r_t, r_c clearance rates from liver trabecular and cortical bone, respectively. f-factors are the corresponding fractions of the total flow F directed back to resting (f_{rt}, f_{rc}) and forming (f_{ft}, f_{fc}) bone surfaces, to liver (f_l) and excretion (f_e).

good agreement with estimated rates of bone resorption. The blood flow of Pu declined from an initial 0.15% of the injected activity/day to 0.05%/day after 3000 days. It was also shown that after about 1000 days in cancellous bone practically all surface deposits should be "secondary", i.e. generated by recirculated Pu-239, and about one third of the activity should be in diffuse deposits. In cortical bone, secondary surface deposits and diffuse labels were found to be insignificant.

Radiation dose factors for human and dog osteons and plane trabecular bone surfaces were calculated by means of a Monte Carlo simulation of alpha-particle decays in bone volume or on bone surfaces. The calculations revealed that within Haversian canals of beagle dogs, having a diameter of 30 µm, dose rates for equal concentrations are significantly (50% and more) enhanced compared to human osteons (70 µm diameter) or plane surfaces. Tables were given that allow dose factors to be derived for the relevant range of alpha-particle energies, diameters and target distances. Also the contribution of cross-fire to the dose rate was determined for both Pu-239 and Ra-226 and daughters. For Pu-239 decaying at the surfaces of Haversian canals, the percentage of particle energy absorbed in the tissue of the canal increases from 24.5% to 44.3% for diameters ranging from 20-80 µm. With respect to the whole tissue irradiated, dose factors in Haversian canals of beagles are about three times and in humans about two times higher than for the same concentration at trabecular surfaces. This difference is smaller for locations close to the surface.

Non-uniformity and relative distribution factors were derived from the dose factors of Ra-226 and Pu-239. The non-uniformity factor of Pu-239 (Dose rate to bone lining cells/average skeletal dose rate) is significantly larger (27.2) in humans than in beagle dogs (14.0). For Ra-226 the non-uniformity factor is close to one. The relative distribution factor (non-uniformity factors Pu-239/Ra-226) is 23 for humans and 15.6 for beagles.

In another study a variety of morphometric, histomorphometric and dosimetric parameters such as the mass of bone and marrow, bone surface areas, percentage of bone volume, surface concentrations of Pu-239 etc. were derived for the beagle skeleton. The total bone surface of the beagle was estimated as 2.9 m^2 with 53.7% of the area associated with trabecular and the rest with cortical bone. The initial surface concentrations of Pu-239 after a single injection of 592 Bq/kg body weight were estimated in all major parts of the beagle skeleton. Where direct comparisons were possible, these calculated estimates were in good agreement with autoradiographic measurements. Assuming that the relative risk of tumor induction is proportional to the collective dose to either bone lining cells, or to osteoblasts, the frequency of tumor occurrence was calculated for the different parts of the skeleton and compared to observed frequencies. Both hypotheses yielded approximate agreement with experimental data for different ratios of trabecular/cortical radiation sensitivity, although the differences in some bones were statistically significant.

As the foundation of cell-specific dosimetry in cortical bone, a mathematical model of osteon closure was designed. The model takes into account the essential physiological features of cortical bone remodeling, such as matrix synthesizing activity of osteoblasts, their burial as osteocytes and the elimination of cells. The calculations show that, both in humans and beagle dogs, osteoblast activity steadily decreases during radial closure of the osteon. The model also predicts an initial increase in thickness of osteoid seams up to about 20 µm, and from there a gradual decline to zero. The kinetic behavior of seam thickness was in very good qualitative agreement with measurements. The calculated variation in osteoblast density during osteon closure (Fig. 3) represents very valuable information for the calculation of collective doses in a detailed model of cell-specific dosimetry in forming osteons.

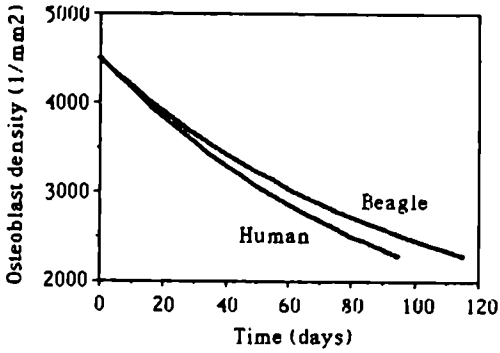


Fig. 3 Areal density of osteoblasts in a closing osteon as a function of time since the onset of bone formation.

Discussion

The slow release of Ra-226 from the proximal ulna and the relatively fast release from the lumbar vertebra indicated that retention in individual bones is correlated with bone turnover rates. The proximal ulna and the lumbar vertebra are representative for skeletal sites of low and high turnover, respectively. Thus, in spite of the lower uptake of Ra-226 by low turnover sites, cumulative radiation doses in low turnover sites over extended periods may be comparable or even exceed those of high turnover sites. This is true in particular for cortical bone and thus may explain the predilection of Ra-226-induced tumors to cortical sites. In addition calculated dose factors for the Haversian systems of beagles show that radiation dose rates in these systems are significantly elevated compared to equal concentrations of Ra-226 at trabecular surfaces. Radiation induced non-neoplastic changes at the highest injection level in the University of Utah beagle study underline the importance of extending the present experiment by inclusion of lower dose levels.

Kinetic modelling of the microdistribution of Pu-239 indicates that the common assumption of a more or less complete transformation of the surface labels of a surface-seeker into diffuse volume labels may have to be revised. It was shown that the fraction of deposited activity which is in diffuse labels increases to the asymptotic limit:

$$\frac{1}{1 + \frac{\sigma_o}{q_{fr}\,\sigma_f}} \qquad (1)$$

where σ_o is the mean interval of quiescence at a specific surface location, σ_f is the formation interval and q_{fr} is the affinity ratio forming/resting surfaces of the radionuclide. According to Eq. 1 a nearly complete volumization would require turnover rates and affinity ratios exceeding 200%/year and 10, respectively. Such values are unlikely in the beagle dogs and even more so in humans. Thus a significant fraction of the skeletal deposits of a surface-seeker will always exist as surface labels. This has important implications for any kind of risk analysis and risk comparison.

The good agreement between toxicity ratios Pu-239/Ra-226 determined in the Utah beagle colony (16.6) and the relative distribution factors based on Monte Carlo calculations of dose factors (14.5-15.6) suggests, that the underlying assumptions for the simple risk model outlined above are not entirely wrong. The success of this approach also indicates that refined models, taking into account the dynamic change of labels, may even do better. Thus the ultimate goal of describing the local distribution, toxicity and relative and absolute risk of bone-seeking alpha-emitters by means of a comprehensive model, based on autoradiographic, histomorphometric and radiochemical data, seems to be reachable. The progress made during the present contractual period constitutes a solid foundation.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Radiobiology Division of the University of Utah. Salt Lake City, Utah 84112, U.S.A.. Prof. W.S.S. Jee

V. Publications:

E. Polig, W.S.S. Jee. Cell-specific radiation dosimetry in the skeleton. Calcif. Tiss. Int. 39:119-122 (1986).

E. Polig, F.W. Bruenger, W.S.S. Jee. Quantitative autoradiography of 226-Radium in bone: I. The measurement technique. Rad. Prot. Dosimetry 16,205-211 (1986).

E. Polig, W.S.S. Jee. Bone age and remodeling: A mathematical treatise. Calcif. Tiss. Int. 41: 130 (1987).

E. Polig. Quantitative autoradiography of 226-Ra in bone: II. Data analysis. Rad. Prot. Dosimetry 19: 139 (1987).

E. Polig. Kinetic model of the distribution of 239-Pu in the skeleton. Health Phys. ... in press.

E. Polig, W.S.S. Jee, R. Dell, F. Johnson. Microdistribution and local dosimetry of 226-Ra in trabecular bone of the beagle. Rad. Res. 116: 263 (1988).

E. Polig. Radiation dose factors for alpha-emitters in osteons and some considerations on dose non-uniformity ratios and relative distribution factors. Phys. Med. Biol. ... in press.

E. Polig, W.S.S. Jee. A model of osteon closure in cortical bone. Calcif. Tiss. Int. ... in press.

E. Polig, W.S.S. Jee. Bone structural parameters, dosimetry and relative radiation risk in the beagle skeleton. Rad. Res. ... in press.

E. Polig, W.S.S. Jee. Microdistribution of 226-Ra and resulting radiation dose to the target cells. Brit. J. Radiol. ... in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Physikalisch-Technische
Bundesanstalt (PTB)
Bundesallee 100
D-3300 Braunschweig

Contract no.: BI6-A-012-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. S. Wagner
PTB
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D-3300 Braunschweig

Prof. Dr. R. Jahr
PTB
Bundesallee 100
D-3300 Braunschweig

Telephone number: 592-7010

Title of the research contract:

Investigation and development of neutron spectrometers and investigation and implementation of dose equivalent quantities.

List of projects:

1. Development of neutron spectrometers for radiation protection practice.
2. Realization of dose equivalent quantities for photons and neutrons using microdosimetric methods.
3. Investigation of dose equivalent quantities for individual dosimetry

Title of the project no.: 1
Development of neutron spectrometers for radiation protection practice

Head(s) of project:

Dr. H. Klein, Dr. K. Knauf

Scientific staff:

A. Alevra, Dr. S. Guldbakke, Dr. M. Matzke, Dr. B.R.L. Siebert

I. Objectives of the project:

Development and investigation of neutron spectrometers for use in real radiation fields

a) Development of a well-specified and reproducible multisphere system (Bonner spheres). Experimental determination of the response of Bonner spheres in monoenergetic neutron fields with the aim of establishing a response matrix by intercomparison with calculated responses.
b) Development of a neutron spectrometer using recoil proton proportional counters and an NE213 scintillation detector. Investigation of these systems in monoenergetic neutron fields and in fields with broad energy spectra.

II. Objectives for the reporting period:

The analysis of the Bonner-sphere measurements will be completed. Further measurements with thermal neutrons and radionuclide sources are planned. The measured responses will be compared with theoretical predictions to obtain a consistent response matrix. Various few channel unfolding codes will be tested for use with Bonner sphere spectrometers. The development of a neutron spectrometer with NE213 scintillators is continued. Spectrum unfolding techniques will be compared with time-of-flight spectrometry. Work on the movable system for on-line indication of spectral neutron fluences is continued. The neutron spectrum of the iron-filtered reactor beam and of a Cf252 source together with resonance materials will be examined to achieve a multi-line spectrum for the calibration of proportional counters.

III. Progress achieved:

a) **Bonner Sphere Spectrometer**

In order to obtain reliable fluence response functions for Bonner sphere (BS) neutron detectors, the detailed analysis of the experimental calibration of the four Bonner-sphere sets (NPL, GSF and 2 x PTB) for monoenergetic neutrons between 1 keV and 14.8 MeV was continued, but could not be finally completed. The estimation of the uncertainty of the fluence normalization factors and, particularly, the corrections for inscattering of source neutrons from the target backing and other neutron background required additional experimental (time-of-flight measurements) and theoretical (Monte Carlo simulation) investigations. The analysis of recent measurements at PTB is in progress. Nevertheless, the preliminary experimental data could already be compared with new ANISN calculations which D. Thomas (NPL) made available. The energy dependence of the fluence response of the Bonner spheres is satisfactorily described (Fig. 1) if an energy independent renormalization (± 15 %) is considered, which shows only a smooth dependence on the diameter of the spheres. The resulting response matrix was then used to unfold first measurements in the field (see d). For this purpose, various few channel unfolding codes (SANDII, STAY'SL, MIEKE) were made available by M. Matzke (PTB).

The reliability of the unfolding codes generally applied to date was checked in an international intercomparison. "Experimental" data sets for measurements with 4 resp. 8 BS's were simulated on the basis of realistically synthesized neutron fields and an assumed ideal response matrix. 8 laboratories from 4 European countries participated with 16 different unfolding codes (or variants). While the integral data as neutron fluence and dose equivalent were reproduced within ± 15 %, the spectral

information scattered significantly as to be expected. A detailed report is in preparation.

b) **Spectrometry with NE213 scintillation detectors**
The experimental specification of the light output and resolution functions for three NE213 systems resulted in the calculation of response functions (NRESP code) for monoenergetic neutrons (ISO recommended calibration energies between 1.2 MeV and 19 MeV). Experimental response spectra were satisfactorily reproduced in shape as well as in the absolute scale (the fluence calibrations with a proton telescope yielded an energy independent correction by + 2 %). The response matrix was therefore constructed by means of Monte Carlo simulations at first for the detector system used for external measurements (3.8 cm in height, 3.8 cm in diameter). A modified version of the FERDOR code, already checked at the PTB by comparison with time-of-flight measurements, was available for the unfolding of the pulse height spectra induced by neutrons with energies from 1 to 20 MeV.

c) **Spectrometry with proton recoil proportional counters**
Cylindrical and spherical proton recoil proportional counters were applied for spectrometry of neutrons. During 1988, the calibration facility based on Fe-filtered neutrons from a ^{252}Cf source and installed in 1987 has been extensively used for testing and calibrating proportional counters. The facility is well-suited for this purpose because of its simple use and structured neutron spectrum.

Furthermore, the spectral flux density of the Fe-filtered reactor neutrons as well as of those filtered by a combination of Fe, Al and S was measured. Approaches to compare these spectra with calculated transmission spectra of the filters show good results (Fig. 2).
Two spherical counters filled with H_2 to a pressure of

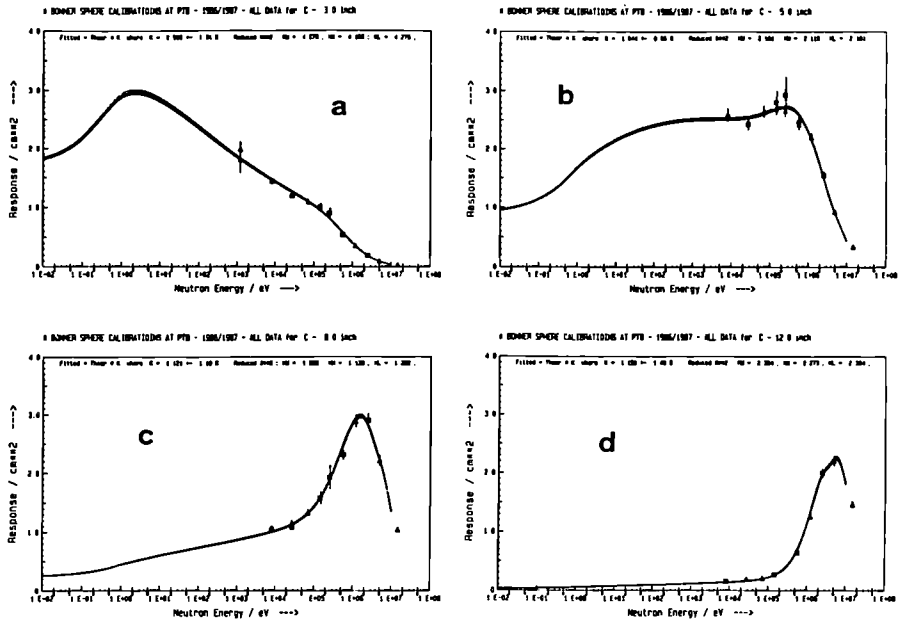
0.93 MPa and 0.29 MPa were employed in external measurements (see section d). For that purpose a transportable system was used and additional efforts have been applied for the unfolding of measured pulse height spectra, when statistical fluctuations could not be ignored.

d) **Application in the field**

Finally, all three types of neutron spectrometers were simultaneously used for the first time in October 88 for external measurements at the national storage for nuclear fuel materials in Hanau. Preliminary analysis of the data obtained by the different systems showed reasonable agreement of the measured neutron flux density distributions in the overlapping energy regions. A first short report is in preparation.

Fig. 1: (a-d) Preliminary experimental calibration data for 4 spheres of the PTB-C-set (diameters 7.62 cm (a); 12.7 cm (b); 20.3 cm (c) and 30.5 cm (d)) compared with ANISN-calculations fitted in absolute scale only. (e) Complete set of response functions for the C-set of Bonner spheres as used for unfolding procedures.

Fig. 2: Comparison of the measured and calculated relative spectral flux density, $\Phi_{E,rel}$, of iron filtered reactor neutrons. Measured spectrum (squares), calculated transmission spectrum (small lines) and calculated transmission spectrum folded with a gaussian and fitted to the measured data (thick line)



* BONNER SPHERE CALIBRATIOINS AT PTB - 1986/1987 - ALL DATA for all C spheres

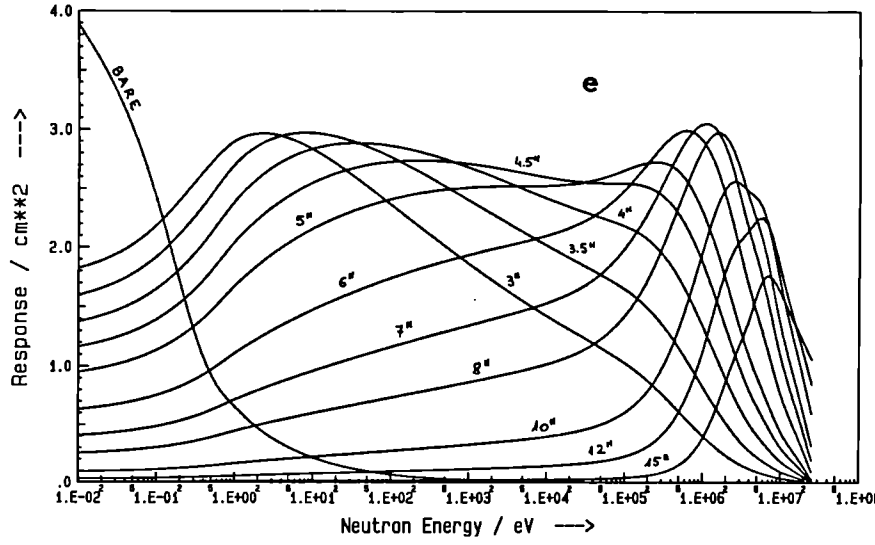


Fig. 1

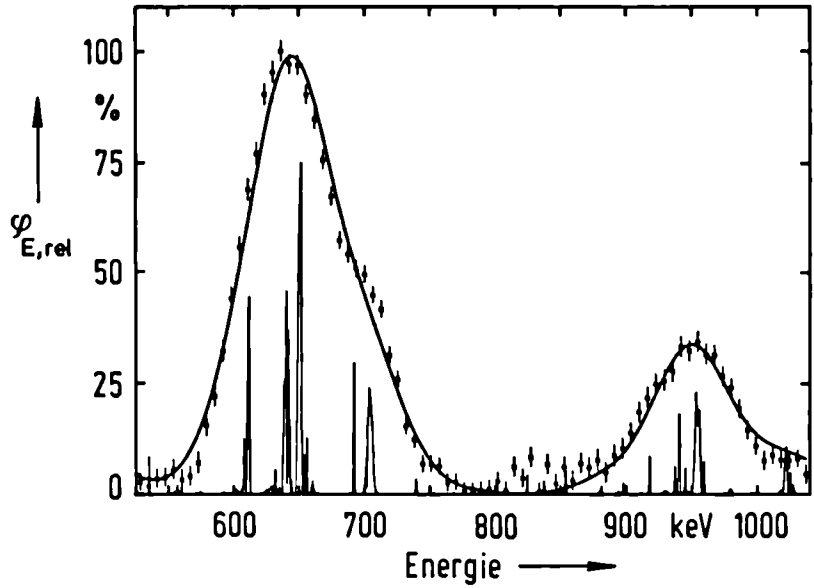


Fig. 2

IV. Objectives for the next reporting period:

The analysis of the calibration measurements of Bonner spheres between 1 keV and 14.8 MeV will be completed. Further measurements with thermal neutrons and radioactive neutron sources (Am/Be, Cf) will be performed at the NPL in 1989.The required electronics of the NE213 spectrometer should be as compact as possible for an easy use in the field. Special requirements for the measurements in restricted areas must be considered. The PC spectrometer used in Hanau will be modified for its use in control areas. A better code for unfolding spectra from `low level' measurements will be developed. The Fe-filter measurements will be further analysed in comparison to calculated transmission functions in order to obtain a good calibration facility for neutron spectrometers.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. J.B. Hunt, Dr. D.. Thomas, Mr. A.G. Bardell
Div. of Rd. Science and Acoustics National Physical Laboratory,
Queens Road, Teddington, Middx, TW11, OLW, UK
Dr. G. Portal, J.L. Chartier, Department de Protection Technique
Commissariat a l'Energie Atomique,
Fontenay-aux-Roses, France
Dr. H. Schraube
Gesellschaft für Strahlen- und Umweltforschung
Institut für Strahlenschutz,
D-8042 Neuherberg, Fed. Rep. of Germany

VI. Publications:

1. A.V. Alevra, M. Cosack, J.B. Hunt, D.J. Thomas and H. Schraube
Experimental Determination of the Response of Four Bonner-Sphere Sets to Monoenergetic Neutrons
Radiat. Prot. Dosim. 23 (1988) 293 - 296

Title of the project no.: 2

Realization of dose equivalent quantities for photons and neutrons using microdosimetric methods

Head(s) of project:

Dr. W.G. Alberts, Dr. G. Dietze

Scientific staff:

Dr. H.J. Brede, Dr. S. Guldbakke, H. Kluge, Dr. U.J. Schrewe, Dr. H. Schuhmacher

I. Objectives of the project:

Investigation of tissue-equivalent low-pressure proportional counters (TEPC) for determining dose equivalent quantities.
Development of a transfer instrument based on a TEPC.
Measurement of dose equivalent in tissue-equivalent spheres.
Intercomparison of instruments for practical radiation protection dosimetry based on tissue-equivalent proportional counters in well-defined monoenergetic and broad neutron fields.

II. Objectives for the reporting period:

Investigation of tissue-equivalent proportional counters (TEPC) in neutron fields below 1 MeV using time-of-flight techniques.
Realization of a transportable data acquisition system with on-line analysis for use with a TEPC-based dosemeter.
Analysis of the second intercomparison of dose-equivalent meters based on microdosimetric techniques.

III. Progress achieved:

1. A transportable data acquisition system

The pulse-height analysis system for tissue-equivalent (TE) and other low-pressure proportional counters (PC) for dosimetric applications requires a special layout of the analog and the digital data processing in order to handle simultaneously detector pulses of a wide dynamic range (10^6:1) and other correlated measurement parameters, e. g. a pulse height proportional to the according time of flight of the detected photon or neutron. The most profitable solution is to process the pulse-height signals of the detectors by different amplification circuits simultaneously and to measure the different pulse-height signals together with further parameters as correlated events with a multiparameter data-acquisition system. A transportable multiparameter data-acquisition system was developed specifically for meeting the requirements of low-pressure proportional counters. Hard- and software components have been almost completed during the present period.

Two coordinated microprocessors which communicate via a standard interface system (CAMAC) were employed. One processor is used as front-end processor for the real-time acquisition and for the preprocessing of the random events and the second one for non-time-critical control tasks and online analyses. As a result of the preprocessing, various data buffer arrays are generated in an independent digital storage device which can also be accessed by the second processor for further data analysis. Further technical details are reported in Ref. 5.

2. Application of time-of-flight techniques below 1 MeV neutron energy

Photon and neutron dose fractions of mixed fields can be distinguished to some extent with proportional counters since the secondary particles, leptons produced by photons and light

ions produced by neutrons, have a different ionisation density along the flight path in the slowing down process. Photons and neutrons are therefore attributed to the lower and the upper part of the pulse-height spectrum measured with tissue-equivalent proportional counters. However, the different pulse-height regions overlap to some extent, especially if low-energy (less than 100 keV) photon and neutron components are present in the field.

As supplementary techniques we have studied a direct separation of photon- and neutron-induced events in fields produced with a pulsed accelerator beam by using their difference in time of flight (TOF). The investigations were carried out in order to obtain a better knowledge on the response of a PC to the separated field components and to improve the photon-neutron separation if TOF techniques can not be applied.

The TOF techniques require a good time-resolution capability of the detectors which depends also on the individual geometrical properties of different counter types and is further influenced by their size, the properties of the counting gas and of the fast timing electronics. The best conditions for a separation of different field components are obtained by proportional counters of Rossi type having a cavity diameter of 13 mm and TE-gas mixtures based on isobutane with a gas pressure simulating a tissue thickness of 2 µm. A deterioration of the timing resolution is caused if the electronic time-trigger signals for the TOF depend on the pulse height. Instead of using an electronic compensation it is more profitable to analyse the frequency distribution of events as function of two parameters, the lineal energy corresponding to the pulse height and the corresponding TOF. The transportable multiparameter system described above (see 1.) is specifically dedicated to perform such an analysis.

Mixed photon-neutron fields with neutron energies below 1 MeV produced by bombarding Li targets with proton beams of 1.94 MeV and 2.23 MeV were the major subject of our investigations in the present period. Some results were reported in Ref. 5, a final report on this subject is in progress. The main results are: 1. The absorbed dose distribution of the photon component indicates the presence of low energy photons. A fitting of the photon spectrum by assuming a distribution as measured for radioactive sources of ^{60}Co or ^{24}Na may therefore underestimate the actual photon dose fraction; 2. The dose distribution of the neutron component within the overlapping region can be described by a function whose shape is independent of the neutron energy between 30 and 144 keV.

A separation of photon and neutron dose fractions could also be achieved for fields of much higher neutron energy provided a high neutron production rate allows to measure in larger distances from the target. The relative photon dose fraction of the PTB's intense Be+d neutron source was determined with a TEPC positioned at a distance of 6 m from the neutron source to be (0.66 ± 0.04) % of the total dose.

3. Second intercomparison of dose-equivalent meters based on microdosimetric techniques

A second series of intercomparison measurements of TEPC prototypes used for area monitoring was performed in late 1987 in order to extend the energy range of the former intercomparison to higher and lower energies and to include further instruments.

Nine systems of seven institutes were irradiated in a thermal, a 24.5 keV and a 144 keV neutron beam produced by filtering neutrons from the FMRB reactor, and neutron fields of 570 keV, 2.5 MeV and 14.8 MeV produced by the Van-de-Graaff

accelerator. ^{60}Co and D_2O-moderated ^{252}Cf radiation sources were also employed.

The results of the measurements (dose and dose equivalent or the respective rates as evaluated by the participants using their individual methods) were divided by the reference tissue kerma free in air or ambient dose equivalent values to yield the kerma responses, R_K, and the dose equivalent responses, R_H, respectively, of the various instruments. The kerma response of most of the systems remains nearly constant with neutron energy decreasing down to 0.57 MeV. A further reduction of neutron energy leads to a decrease in R_K. A similar, but more pronounced trend is observed for the dose-equivalent response with the decrease at low energies. R_H varies by a factor of 2.5 to 16, most systems showing a variation by a factor of 5. Except for one instrument, which shows a minimum of R_H at about 100 keV, the minimum values are observed at 24.5 keV. With the two parts of the inter-comparison a comprehensive set of response data for mono-energetic neutrons in the energy range from thermal neutrons to about 15 MeV and for broad spectra produced by a $^{252}Cf(D_2O)$ source has been obtained (cf. also Refs. 1, 2).

IV. Objectives for the next reporting period:

Completion of the transportable multiparameter system and its employment to complete investigations concerning neutron and photon dose separation in monoenergetic neutron fields based on time-of-flight techniques. Measurement of kerma factors in neutron fields of energies above 30 MeV by separating neutron components of lower energy by their difference in velocity.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. H.G. Menzel et al., Universität des Saarlandes, Fachrichtung Biophysik, D-6650 Homburg/Saar
EURADOS Committee 1 ("Dose equivalent meters based on microdosimetric techniques")

VI. Publications:

1. Alberts, W.G.; Dietz, E.; Guldbakke, S:; Kluge, H.; Schuhmacher, H.:
   Radiation Protection Instruments Based on Tissue Equivalent Proportional Counters: Part II of an International Intercomparison. Report PTB-FMRB-117 (1988)
2. Alberts, W.G.; Dietz, E.; Guldbakke, S:; Kluge, H.; Schuhmacher, H.:
   International Intercomparison of TEPC Systems used for Radiation Protection. Workshop Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection. Schloß Elmau, 1988 (to be published in Radiat. Prot. Dosim.)
3. Pihet, P.; Menzel, H.G.; Alberts, W.G.; Kluge, H.:
   Response of Tissue-Equivalent Proportional Counters to Low and Intermediate Energy Neutrons Using Modified TE-^{3}He Gas Mixtures. Ibid.
4. Menzel, H.G.; Lindborg, L.; Schmitz, Th.; Schuhmacher, H.; Waker, A.J.:
   Intercomparison of Dose Equivalent Meters Based on Microdosimetric Techniques. Detailed Analysis and Conclusions. Ibid.

5. Schrewe, U.J.; Brede, H.J.; Dietze, G.:
Dosimetry in Mixed Neutron-Photon Fields with Tissue-Equivalent Proportional Counters. Ibid.

6. Dietze, G.; Edwards, A.A.; Guldbakke, S.; Kluge, H.; Leroux, J.B.; Lindborg, L.; Menzel, H.G.; Nguyen, V.D.; Schmitz, Th.; Schuhmacher, H.:
Investigation of Radiation Protection Instruments Based on Tissue-Equivalent Proportional Counters.
CEC-Report, EUR 11867 EN (1988)

7. Menzel, H.G.; Dietze, G.; Schuhmacher, H.:
Practical Determination of Dose Equivalent Using Low-Pressure Tissue-Equivalent Proportional Counters.
Radiation Protection Practice, Vol. I, (1988) 308 - 311 (Pergamon Press, Sydney)

8. Dietze, G.; Menzel, H.G.; Schuhmacher, H.:
Measurement of Ambient Dose Equivalent with Tissue-Equivalent Proportional Counters. CEC-Seminar, Juni 1988

Title of the project no.: 3
Investigation of dose equivalent quantities for individual dosimetry

Head(s) of project:

Dr. B.R.L. Siebert

Scientific staff:

Dr. W.G. Alberts, B. Bauer, Dr. S. Guldbakke, Prof. Dr. R. Jahr, H. Kluge

I. Objectives of the project:

Experimental and theoretical examination of individual dosemeters. Investigation of procedures for calibration and evaluation of individual dosemeters in order to achieve compliance with the system of dose limitation for radiation protection. Investigation and intercomparison of appropriate quantities for individual dosimetry (choice of phantom and measurement positions).

II Objectives for the reporting period:

Computational study of the influence of slab phantoms on detector responses and calibration and comparison with the ICRU-sphere and the hollow cylinder phantoms. A new Monte-Carlo program will be used in order to obtain additional confidence in the results. Measurement of the free-in-air and on-phantom response of individual dosemeters at low neutron energies (filtered beams at the reactor). Test of the hypothesis that the on-phantom response may be calculated from measured free-in-air responses and computed albedo fluences. Search for optimal calibration procedures.

III. Progress achieved:

The systematic experimental determination of fluence and dose equivalent responses of thermoluminiscent albedo dosemeters free in air and on various phantoms (slab, cylinder and sphere made of polyethylene) could be nearly completed (see publication No. 1). As compared to the hollow elliptical cylinder (height 70 cm, outer semiaxes 20 and 10 cm, inner semiaxes 8 and 4 cm) one obtains in the case of AP irradiation a lower fluence response on a sphere (radius of 15 cm) and a slightly higher response on a slab (40 x 40 x 15 cm^3). This trend is reversed for lateral irradiation, where the response on the sphere is higher than on the cylinder. This phantom dependence of dosemeter responses found experimentally follows in general the trend predicted by a numerical study (Bauer, B.W., Hollnagel, R., Siebert, B.R.L., Radiat. Prot. Dosim. 23 (1988) 207 - 210). In discussing the problems with the quantities for individual monitoring presented in ICRU Report 39, a different quantity for individual monitoring has been proposed and a metrologically sound procedure for calibrating individual monitors have been presented (see publication No. 2). The directional dose equivalent, H'(10), is defined in the ICRU sphere in an expanded field. It is proposed to drop the requirement of expanding the field. This leads to a new dose equivalent quantity, $H^i(10)$. This quantity is numerically identical to H'(10) in fields from radiation sources far away. $H^i(10)$ is also defined for sources close to the phantom or body, whereas H'(10) is not. Using numerical results from calculations for a slab phantom it could be shown, that the numerical values of the respective fluence-to-dose equivalent conversion factors are practically the same as for $H'(10)/\Phi$ for distances as close as 25 cm.

A simple formalism (see publication No. 3) has been employed to average data on neutron fluence-to-directional dose equivalent conversion factors, $h'(10;E_n,\alpha)$, for various neutron energies, E_n, and angles of radiation incidence, α, as given by three different research groups. $h'(10;E_n,\alpha)$ is represented as the product of the neutron fluence-to-ambient dose equivalent conversion factors, h^* and a factor given by a simple polynomial expension using only 15 pairs of coefficients for describing the angular dependence in the neutron energy region from thermal up to E_n = 20 MeV. Good agreement between the fit, the input data and more recent data which were not included in the evaluation has been obtained. It is shown that the ICRU sphere may serve as the defining phantom for operational quantities for individual monitoring, restricted, however, to irradiation from the frontal half space.

IV. Objectives for the next reporting period:

1. Supplementary measurements, especially using bare and D_2O-moderated ^{252}Cf sources.
2. Completion and application of a Monte Carlo programme for predicting the response of dosemeters on phantoms if their fluence response free in air in known.
3. Summary report on the experimental results.
4. Theoretical interpretation of the results and discussion of appropriate quantities for individual dosimetry in neutron fields.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

G. Portal, CEN, Fontanay-aux-Roses, France and
E. Piesch, KfK, Postfach 36 40, Karlsruhe, Germany

VI. Publications:

1. B.W. Bauer, W.G. Alberts, B. Burgkhardt, S. Guldbakke, R. Medioni, E. Piesch, G. Portal, B.R.L. Siebert
Energy and Angle Dependence of and Phantom Influence on Readings of Neutron Individual Dosemeters: First Result of Experiments (accepted for publication in Radiat. Prot. Dosim.)
2. R. Jahr, B.R.L. Siebert, W.G. Alberts
Operational Quantities and Calibration Procedure for Individual Monitoring
(accepted for publication on Radiat. Prot. Dosim.)
3. B.R.L. Siebert, A. Morhart
A Proposed Procedure for Standardizing the Relationship between the Directional Dose Equivalent and Neutron Fluence (accepted for publication in Radiat. Prot. Dosim.)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

University of St.Andrews
College Gate
St.Andrews
GB Fife KY16 9AJ

Contract no.: BI6-A-024-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. D.E. Watt
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University of St.Andrews
North Haugh
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Telephone number: 334-75851

Title of the research contract:

Specification and measurement of radiation effectiveness.

List of projects:

1. Specification and measurement of radiation effectiveness.

Title of the project no.: Specification and Measurement of Radiation Effectiveness

Head(s) of project: Dr D E Watt

Scientific staff: E B Saion

I. Objectives of the project:

To develop a microdosimeter for the measurement of dose equivalent of intermediate energy neutrons in mixed fast neutron and γ-ray fields.

II. Objectives for the reporting period:

To complete laboratory trials with the tissue-equivalent version of the microdosimeter; to explore methods for fabricating the thin (~ μm) plastic dividing wall; to determine correction factors for the unwanted contribution by fast neutrons to the low energy recoil event spectrum generated by intermediate energy neutrons; to devise necessary computer programmes for analysis of data and theoretical calculations; to set-up field trials.

III. Progress achieved: The test with the prototype non-tissue equivalent co-axial double cylindrical proportional counter (ref 1) with a laboratory neutron source has been completed. The results confirm the applicability of the dosimeter for discrimination in favour of intermediate energy neutrons in mixed radiation fields.

To facilitate its operation, an improved coincidence/anti-coincidence unit has been built with a special feature to remove the effect of low-voltage noise pulses.

Upon completion of the above tests, the prototype version in tissue-equivalent materials was designed, constructed (fig 1) and commissioned for measurement of event size spectra, produced by a selectable neutron energy band determined by the thickness of the common dividing wall. Although thicknesses of about 2 μm were practical with conducting melinex (selectively sensitive to neutrons < 100 keV) it was found impossible to produce satisfactory walls with A-150 TE plastic at that thickness which were sufficiently homogeneous and conducting. Improved fabrication techniques are required. Consequently the counter was constructed with a 16 μm self-supporting wall of A-150 TE plastic making it selectively sensitive to neutrons with energy < 850 keV in mixed fields. Good gamma ray discrimination is obtained.

The operational characteristics of the new co-axial cylindrical microdosimeter have been determined in a series of experiments with neutron and photon sources. Field trials with reactor neutrons have begun and will be completed in 1989.

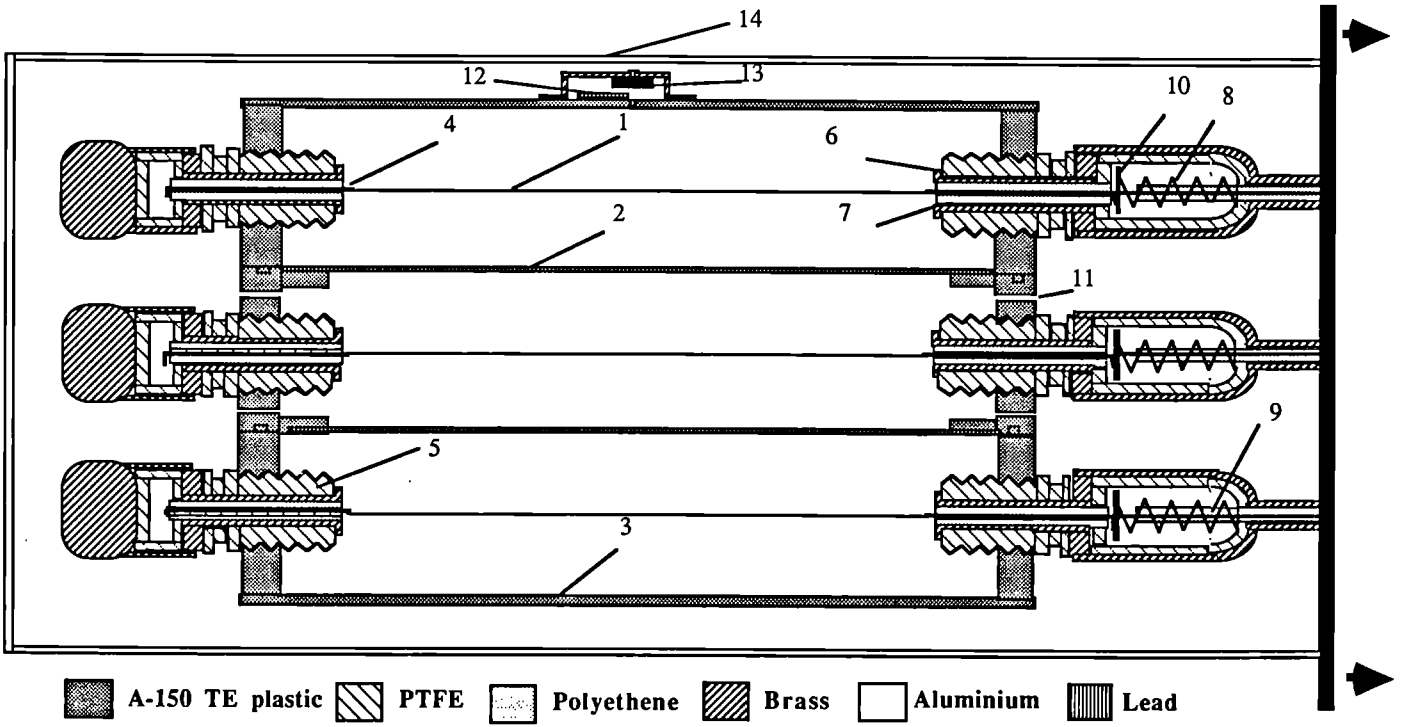

Fig. 1 Schematic drawing of the Co-Axial Double Cylindrical TEPC; 1. Anode, 2. Dividing Wall, 3. Outer Wall, 4. Copper Wire, 5. Insulator, 6. Guard Tube, 7. Insulator Tube, 8. Spring, 9. Co-Axial Cable, 10. Contact Plate, 11. Hole for Gas Filling, 12. Lead Shutter, 13. Alpha Internal Source, 14. Electrostatic and Vacuum Canister.

IV. Objectives for the next reporting period: To complete field trials with the microdosimeter. The computer programme for extraction of quality factor and ambient dose-equivalent will be tested and prepared as a ROM. Design requirements for a portable device with built-in logarithmic amplifiers and information technology will be explored to complete the project.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr P Colautti, Institute of Nuclear Physics, Legnaro, Padova, Italy.

VI. Publications:

(1) Saion E B and Watt D E (1988). Microdosimetry of intermediate energy neutrons in fast neutron fields. Radiat Protect Dos, 23, 1-4, 265-268.

Other related publications:

(2) Watt D E (1988). Absolute Biological Effectivess of Neutrons and Photons. Radiat Protect Dos 23, (1/4), 63-67.

(3) Al-Kazwini A T, Cunningham J W and Watt D E (1988). Damage by nuclear elastic scattering (NES) - forty years on. Int J Radiat Biol 53, (4), 683-685.

(4) Watt D E (1989). On absolute biological effectiveness and unified dosimetry. J Radiol Protect 9, - (in proofs).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

European Radiation Dosimetry Group
EURADOS/CENDOS
Radiobiological Institute TNO
P.O.Box 5815
NL-2280 HV Rijswijk

Contract no.: BI6-A-026-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.A. Dennis
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Telephone number: 235/831600/2221

Title of the research contract:

Collaboration on research and development concerned with the methodology and data of radiation dosimetry.

List of projects:

1. Development and implementation of microdosimetric instruments and methods for radiation protection.
2. Skin dosimetry and surface contamination monitoring
4. Dissemination and development of computer programs for dosimetric problems. ('Numerical dosimetry')
5. Basic physical data and characteristics of radiation protection instrumentation.
6. Assessment of internal dose.

III. Progress achieved:

The international intercomparison of prototype area monitors based on microdosimetric techniques jointly organised by EURADOS and Physikalisch-Technische Bundesanstalt (PTB), Braunschweig has been published by the Commission of the European Communities and the results of the second part were reported in a PTB Report (1) (PTB-FMRB-117), 1988) and in a paper (2) presented at the Workshop at Schloss Elmau (see below). A detailed analysis of the results obtained by participants from seven European Institutes (CEA, Fontenay-aux-Roses, CEN Grenoble, PSI (formerly EIR) Wurenlingen (CH), KFA Julich, NIRP Stockholm, University of Leeds, University of Saarland, Homburg) and conclusions and recommendations are provided in a report prepared by members of the Committee and presented at the Workshop at Schloss Elmau.

In view of the state of development of the prototype instruments using tissue equivalent proportional counters (TEPC), the intercomparison was more a research activity than an intercomparison of well established instruments with regard to their technical aspects. The aim was primarily to determine the neutron energy dependence of the dose equivalent response (and absorbed dose response) and to study the influence of the parameters counter geometry, data processing, calibration and evaluation procedure on these responses. The analysis of the results took advantage of the fact that several of the participating systems provided the measured microdosimetric spectra. Details of the results and their analysis can be found in the references and reports given in the list of publications. In summary, it may be concluded that TEPC based area monitors using the pulse height mode or the variance technique can be made to have a dose equivalent response which varies less with neutron energy than existing radiation protection instruments. The decrease of the response below several 100 keV neutron energy is due in equal parts to the deterioration of the TEPC as a LET spectrometer and to differences in the radiation transport processes in the ICRU sphere and in the detector. Improvement of the dose equivalent response appears possible by optimising counter geometry (counter size, wall thickness, simulated diameter), counter materials (wall, gas composition) and evaluation procedures. The sensitivity of TEPC area dose meters can be made to be adequate for all operational conditions of practical interest by choosing the appropriate detector size. The intercomparison has shown that a 12.5 cm diameter TEPC has the same sensitivity as a conventional moderator-type dosemeter. The intercomparison has documented the excellent diagnostic properties of TEPC area monitors which are expected to be useful in unknown radiation fields. Finally, the analysis has shown that the measurement of ambient dose equivalent with a TEPC requires that the conventional calibration procedure in terms of lineal energy is replaced by a calibration procedure in terms of the quantity to be measured, for example in the radiation field of a D_2O moderated ^{252}Cf reference source.

A workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection was organised together with the CEC and Ges. fur Strahlen- und Umweltforschung (GSF), Munich, at Schloss elmau (FRG), 18-20 October 1988. The workshop was attended by some 50 scientists from Europe and North

Title of the project no.:

1. Development and Implementation of Microdosimetric Instruments and for Radiation Protection

Head(s) of project:

H G Menzel

Scientific staff:

A A Edwards, J Booz, G Dietze, G H Hartmann, L Lindborg, A Marchetto, V D Nguyen, H Paretzke, T Schmitz, H Schuhmacher

I. Objectives of the project:

The development of dose equivalent meters based on microdosimetric techniques for use in area monitoring, individual monitoring and as transfer instruments.

II. Objectives for the reporting period:

To analyse the results of the second part of the intercomparison of dose equivalent meters based on microdosimetric techniques and to prepare a report based on this intercomparison. To prepare a report summarising both parts of the intercomparison and providing conclusions and recommendations. To organise, together with CEC and GSF, Neuherberg a Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection at Schloss Elmau (FRG), October 18-20 1988. To continue energy deposition calculations for neutron radiation, (benchmark calculations, development of reference code) and calculations for the response of TEPC to photons.

America. In spite of the special topic of the meeting a large number of experts in the general field of radiation protection dosimetry were attracted. The general impression at the Workshop was that TEPC area dosemeters may be an interesting complement to existing monitors mainly because of their additional diagnostic capacities. The final decision on their routine applicability may depend critically on economical aspects. In addition to the presentation and discussion of the TEPC intercomparison topics such as specification of radiation quality, general requirements for the performance of dose equivalent meters in area and individual monitoring and space radiation dosimetry were covered by invited and proferred papers and extensive discussions. The proceedings of the Workshop will be published in Radiation Protection Dosimetry.

There was some progress in energy deposition claculations for neutrons and photons. Results of these calculations may be available in 1989.

References

(1) Alberts, W G, Dietze, G, Guldbakke, S, Kluge, H, Schuhmacher, H. Radiation Protection Instruments Based on Tissue Equivalent Proportional Counters: Part II of an Internatimonal Intercomparison. Report PTB-FMRB-117, Braunschweig (1988).

2. Alberts, W G, Dietze, E, Guldbakke, S, Kluge, H, Schuhmacher, H. International Intercomparison of TEPC Systems Used for Radiation Protection. Presented at the Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection, Oct. 1988, Schloss Elmau and to be published in Rad. Prot. Dos.

IV. Objectives for the next reporting period:

To publish the proceedings of the Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection. To investigate the feasibility of TEPC dosemeters in fields other than area monitoring (individual monitoring, transfer instruments, space dosimetry). To continue energy deposition calculations for neutrons and photons.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

CEN, Fontenay-aux-Roses
CEN, Grenoble
DKFZ, Heidelberg
GSF, Neuherberg
KFA, Julich
NIRP, Stockholm
NRPB, Chilton
PTB, Braunschweig
University of Leeds
Universitat des Saarlandes, Homburg (Saar)

VI. Publications:

Dietze, G, Booz, J, Edwards, A A, Guldbakke, S, Kluge, H, Leroux, J B, Lindborg, L, Menzel, H G, Nguyen, V D, Schmitz, Th, Schuhmacher, H. Intercomparison of Dose Equivalent Meters Based on Microdosimetric Techniques. Radiat. Prot. Dosim. 23, 227-234 (1988).

Dietze, G, Edwards, A A, Guldbakke, S, Kluge, H, Leroux, J B, Lindborg, L, Menzel, H G, Nguyen, V D, Schmitz, Th, Schuhmacher, H. Investigation of Radiation Protection Instruments Based on Tissue Equivalent Proportional Counters. Results of an EURADOS Intercomparison, Commission of the European Communities, EUR 11867(EN) Luxembourg, (1988).

Menzel, H G, Lindborg, L, Schmitz, Th, Schuhmacher, H, Waker, A J. Intercomparison of Dose Equivalent Meters Based on Microdosimetric Techniques: Detailed Analysis and Conclusions. Presented at the Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection, Oct. 1988, Schloss Elmau and to be published in Radiat. Prot. Dosim.

Title of the project no.:

2. Skin Dosimetry and Surface Contamination Monitoring

Head(s) of project:

P Christensen

Scientific staff:

J Böhm, T O Marshall, M Charles, J Patau, Y Herbaut, E Piesch, J R Harvey, D Regulla, M Heinzlemann, M J Rossiter, H Julius

I. Objectives of the project:

The evaluation of exposures to beta and low energy photon radiations and the development of appropriate techniques and methods for their measurement.

II. Objectives for the reporting period:

To finish the preparation of a review document on dose rate meters for skin dose measurements and to start the preparation of a review document concerned with surface contamination monitoring. To intercompare different computer programs for performing transport calculations concerned with low energy photon radiation. To continue the study of the importance of problems of skin dosimetry and surface contamination monitoring. To perform benchmark intercomparison of computational methods for beta radiation dosimetry. To intercompare the measurement of dose rates from ^{147}Pm sources carried out at different laboratories. To continue the evaluation of the biological effectiveness of low-penetrating radiations in co-operation with EULEP. To co-operate with Directorate General 12 in the planning and organisation of a workshop on skin dosimetry and surface contamination monitoring.

III. Progress achieved:

The review document on dose-rate meters for skin dose measurements is expected to be ready in 1989.

Data on transport and dose deposition of electrons and low energy photons in tissue equivalent materials have been evaluated from Monte Carlo calculations made at CPA, Toulouse, PTB, Braunschweig and KFZ, Julich. At CPA, calculations have been made for electrons with energies below the threshold for electronic excitation, ie, below 10 eV. The work was carried out in co-operation with the University of Sherbrook, Canada and results from the work will be presented at the Tenth Symposium on Microdosimetry, Rome, 21-26 May 1989. At PTB, data were evaluated for absorbed dose in the ICRU sphere from exposures to low energy photons for a number of energies down to 2 keV. Data have been evaluated for depths of 0.07 mm, 3 mm and 10 mm, respectively. At KFZ, similar calculations were made for K X-rays (5-10 keV) from different radionuclides that are present in the working place. Results from the calculations by PTB and KFZ will be published in 1989.

Programmes have been further planned and preparations have been made for benchmark experiments testing the validity of computer programs for determining dose rates from an extended, 8 cm diameter, $^{90}Sr/^{90}Y$ source and particulate ^{60}Co sources of different sizes. The measurements of dose rates from the $^{90}Sr/^{90}Y$ source will take place at PTB, Braunschweig and measurements of the ^{60}Co sources at CEGB, Berkeley and CEN, Grenoble. The calculations will be made at CPA, Toulouse. Results from the experiments will be available during 1989.

In FRG extensive nation-wide programmes concerned with investigations of the importance of beta and low energy photon radiation in workplaces of the nuclear industries have been initiated. Dose-rate measurements using ionisation chambers were carried out by GSF, Neuherberg and KFZ, Julich at a number of nuclear facilities. Different filters were used to obtain information on the energy spectrum of the radiations. Results from these measurements will be published in 1989. At KFK, Karlsruhe, thermoluminescence detectors have been used to evaluate data on depth dose distributions for exposures in certain areas of a nuclear power station and furthermore to interpret the individual doses from exposures in mixed beta-photon radiation fields in areas for the production of low-enriched uranium (see publications below). The Committee will study the results from the workplaces in FRG and consider the possibility of performing similar investigations in other countries.

Comparative measurements of dose rates from four, equal, 4 cm x 4 cm, ^{147}Pm sources at different laboratories (NRPB, PTB, Fontenay-aux-Roses, and Riso) are in progress. Some of the sources have been exchanged between the laboratories. Results from the experiments will be evaluated during 1989.

The evaluation of data on the biological effectiveness of low-penetrating radiation has continued in co-operation with EULEP. A Task Group set up by ICRP to deal with these problems expects to have a report ready for the ICRP Main Committee in 1989.

IV. Objectives for the next reporting period:

To finish the preparation of a review document on dose rate-meters for skin dose measurements. To continue the use of computer codes to evaluate dose data for exposures to beta and low energy photon radiation and to perform benchmark intercomparisons of computational methods for beta radiation dosimetry. To continue the study of the importance of problems of skin dosimetry and surface contamination monitoring. To perform comparative measurements of dose rates from ^{147}Pm sources carried out at different laboratories. To continue the evaluation of the biological effectiveness of low-penetrating radiations in co-operation with EULEP. To co-operate with Directorate General 12 in the planning and organisation of a workshop on skin dosimetry and surface contamination monitoring.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

RNL, Riso
PTB, Braunschweig
GSF, Neuherberg
CEN, Grenoble
KFA, Julich
NPL, Teddington
NRPB Chilton
SPEE/LMR, Grenoble
CEGB, Berkeley
RSO TNO, Arnhem
KFZ, Karlsruhe
CPA, Toulouse

VI. Publications:

Regulla, D F. Beta fields relevant for radiation protection. In: Beta Dosimetry. Fifth Information Seminar on the radiation protection dosemeter intercomparison programme. Bologna, 25-27 May 1987. Luxembourg, 1988 (EUR 11363 EN) pp.165-176.

Burgkardt, B, Piesche, E. Untersuchungen des Beta-Photonen-Mischstrahlungsfeldes im Anlagenbereich des Dampferzeugers im Kerkraftwerk Obrigheim, KfK 4456, Oktober 1988.

Piesch, E, Burgkardt, B, Anton, R, Rudolph, W, Schafer, R, Guldner R. Interpretation der Personendosen in Beta-Photonen-Mischstrahlungsfeldern bei der Fertigung von Brennelementen aus niedrig angerelchertem Uran fur Leichtwasserreaktoren, KfK 4194, December 1988.

Christensen, P, Bohm, J, and Francis, T M. Measurement of absorbed dose to tissue in a slab phantom for beta radiation incident at various angles. In: Beta Dosimetry. Fifth Information Seminar on the radiation protection dosemeter intercomparison programme. Bologna, 25-27 May 1987. Luxembourg, 1988 (EUR 11363 EN) pp.39-75.

Title of the project no.:

4. Dissemination and Development of Computer Programs for Dosimetric Problems ('Numerical Dosimetry')

Head(s) of project:

B R L Siebert

Scientific staff:

M Buxerolle, J L Chartier, P Dimbylow, B Grosswendt, K G Harrison, G Hen, D J Thomas, A J Wittmann
G Burger (Sponsor), A Alevra (Consultant)

I. Objectives of the project:

To disseminate information about computer programs developed in Europe and America for dosimetric and shielding problems by collecting information about existing programs and where necessary testing and evaluating them.

II. Objectives for the reporting period:

1. Discussion and publication of the first step of the intercomparison of unfolding codes for Bonner spheres.
2. Inclusion of experimental variances and covariances in the intercomparison of unfolding codes.
3. Survey on the need for calculation for photon dosimetry.
4. Intercomparison of benchmark calculations for Bonner spheres.
5. Supplementary calculations for Project 1.

III. Progress achieved:

The intercomparison of unfolding codes for Bonner spheres demonstrated clearly that the use of codes as 'black boxes' is not possible. Different participants using the same code (eg, STAYSL or SAND) obtained quite different results as far as the spectra are concerned, although the integral quantities such as absorbed dose or dose equivalent could be reproduced within acceptable uncertainties by all participants. The a priori information to be used in such quite under-determined unfolding problems is of prominent importance, therefore codes should be used that allow a specification of the covariance matrix of the unfolded spectra. This, however, (see objective 2) had to be postponed as the analysis of the discrepancies of spectral unfolding could not be completed.

A survey on the need for calculations for photon dosimetry that especially transport calculations of low energy photons pose problems, as the choice of reaction mechanisms to be included is quite difficult.

Of great interest in the aftermath of Chernobyl is the problem of correctly calculating the dose due to external radiation from Cs contaminated soil. This problem has been discussed and a study will be performed by committee members.

New experimental results on response functions of Bonner spheres still exhibit discrepancies with existing calculations. One possible reason for these discrepancies is the effect of the bonding state of polyethylene on thermal cross sections. The newest version of an analytical code for computing charged particle slowing down spectra and the response of TEPC counters for neutrons (Coyne, et al, NIST, Washington, DC) has been obtained. Versions for VAX (DEC) and CDC computers are available upon request.

A procedure for standardising the relationship between the directional dose equivalent and neutron fluence has been proposed and accepted for publication in Rad. Prot. Dos.

IV. Objectives for the next reporting period:

1. Report on the intercomparison of unfolding codes for Bonner spheres measurements.
2. Benchmark study on response functions for Bonner spheres.
3. Numerical study of external radiation dose from Cs ground contamination.
4. Definition and implementation of benchmark problems for photon and electron transport.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

AERE, Harwell
CEN, Cadarache
IKE, Stuttgart
NRPB, Chilton
CEA, Fontenay-aux-Roses
GSF, Munich
NPL, Teddington
PTB, Braunschweig

VI. Publications:

No publications in this period.

Title of the project no.:

5. Basic Physical Data and Characteristics of Radiation Protection Instrumentation

Head(s) of project:

J J Broerse, J R Harvey, K G Harrison

Scientific staff: 1. Ionization Chambers: H J Brede, J J Broerse, G Dietze, S Guldbakke, V D Haigh, V E Lewis, D R Schlegel-Bickman, H Schraube, U J Schrewe, J Zoetelief. 2. Track-Etch Detectors: D T Bartlett, J-L Decossas, K G Harrison, R A Hollnagel, J R Harvey, L Lembo, R Medioni, E Piesch, H Schraube, L Tommasino, J-C Varielle, G Zapparoli

I. Objectives of the project:

The collection and evaluation of physical data relevant to the assessment of the biological effects of ionizing radiations and to the assessment of occupational and environmental exposures of the population of the European Communities.

II. Objectives for the reporting period:

1. The characteristics of Mg/Ar ionisation chambers will be studied in photon and neutron fields as a joint project between PTB, NPL and TNO with an emphasis on the relative neutron sensitivity. The discrepancies in the k_u values of a series of chambers of the same design will be investigated to resolve this problem in the us of Mg/Ar chambers.
2. A joint irradiation of track-etch detectors will be carried out with participating laboratories from Europe and North America. Attempts will be made to pool information on available plastics for track-etch detection with an emphasis on quality control, background, ageing and environmental effects.

III. Progress achieved:

1. Ionisation chamber studies:

The k_U-values of the Mg/Ar chambers of five of the centres involved in this work were measured in the same neutron fields over a range of energies using a standardised procedure.

Measurements were made at PTB in January/February, 1988 using the Van de Graaff to produce mono-energetic neutron fields with energies of 5, 14.8 and 19 MeV and the cyclotron for 8 MeV. For each field the responses of four of the Mg/Ar ionisation chambers were measured for both positive and negative polarity applied EHT's. Considerable time and effort were devoted to measuring the leakage currents. These data have been analysed and show:

(a) For 3 chambers the ratio of positive to negative response varies with energy and is higher than for the fourth chamber for which it is constant;
(b) Regarding the mean responses, the chambers fall into two pairs, the difference between them being around 30%, 15%, 7% and 2% at 5, 8, 14.8 and 19 MeV respectively.

For each neutron energy a proton recoil telescope was used to measure neutron fluence and time-of-flight techniques were used for spectral measurements, enabling the neutron dose to be derived. The photon dose components were measured using an energy-compensated GM-counter (3). When the analysis of these data is completed it will be possible to determine the k_U-values, applying the expression for the response

$$R_U = D_G + k_U . D_N .$$

Further measurements were made with a tissue-equivalent (A150 plastic) ionisation chamber and a tissue equivalent proportional counter (TEPC). The measured k_T-values for the former will be compared with those calculated using ancillary data. The TEPC measurements of neutron and photon dose components will be compared with those obtained by the fluence plus GM-counter techniques.

2. Track-etch detectors:

Problems have been identified in maintaining consistent background track registration levels in the plastics Cr-39 and CN-38. A programme of collaboration between laboratories has been started that will identify differences between laboratories and the desirability of a research programme on this specific aspect of the track-etch systems has been identified.

A further programme of joint irradiations took place in November 1988, using monoenergetic-neutron and californium-fission neutron sources at GSF and PTB. For these irradiations the dosemeters were exposed on PMMA cuboid phantoms. Further irradiations are planned early in 1989.

IV. Objectives for the next reporting period:

1. The data obtained from the ionisation chamber studies in 1988 will be analysed and further measurements undertaken to resolve an anomalous wall attenuation effect. Results of a recent BIPM intercomparison will be analysed for differences in k_q-values. The need for further experimental work will be examined, possibly using a 14.7 MeV neutron field at NPL. The results of the past work will be published. The case for a European calibration service will be considered.
2. The survey of background track registration levels in track-etch materials will be published and also the results of the joint irradiation experiments. A calculation of albedo neutron spectra from anthropomorphic phantoms will be undertaken. The development of a programme for The investigation of the causes of variability in background track registration levels will be considered.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

BIPM, Paris
GSF, Neuherberg
PTB, Braunschweig
NPL, Teddington
TNO, Rijswijk
AERE, Harwell
NRPB, Chilton
CEA, Fontenay-aux-Roses
CEGB, Berkeley
ENEA, Bologna
ENEA, Rome
KfK, Karlsruhe
Limoges University

VI. Publications:
No publications in this period.

Title of the project no.:

6. Assessment of Internal Dose

Head(s) of project:

J A B Gibson

Scientific staff:

Full Members: P J Darley, Miss F A Fry, K Henrichs, R Kunkel, J Piechowski, R Roth, H Schiefendecker

Corresponding Members: R L Kathren, S Suga, R G Thomas, C Wernli

I. Objectives of the project:

Preparation of guidance on the interpretation of monitoring data relating to internal exposures of radiation workers and the implementation of ICRP recommendations on this topic within Europe. The objective will be achieved by the pooling and exchange of information and in comparing operational experience.

II. Objectives for the reporting period:

To continue a programme of work to: develop computer models for excretion analysis; interpret data from air sampling, bioassay and in-vivo monitoring; establish availability of autopsy data; consider compatibility of dose records; exchange information. Specifically in 1988 the objectives were:
(a) List elements of interest in RP.
(b) Proposals for stable element metabolic studies.
(c) Consider models for elements identified in (a).
(d) Identify sources of autopsy data in Europe.
(e) Compare dose record formats in Europe.
(f) Prepare a database for metabolism and models.
(g) Produce a list of laboratories assessing internal dose.

III. Progress achieved:

Elements of interest in radiological protection have been identified with an indication of both the current state of knowledge of human biokinetics and of priorities for future work. Proposals for stable isotope metabolic studies have been made (Roth, et al, 1988) and include Co, Sr, Y, Zr, Nb, Ru, Ba, Ce; the actinides could be studied using Ba and La as analogues. The review of biokinetic models would be continuing action but avoiding a repetition of the work of ICRP, NCRP and EULEP (a simple computer program is available for the ICRP models for ages 1 yr, 10 yr and adults). There are particular problems for models for certain compounds of nuclides viz, H and C and for the detailed metabolism of Na, Fe, Se, Te, Tc and Cs; particularly for foetal transfer.

The availability of autopsy data was limited in Europe by comparison with that achieved in the USA through their Uranium and Transuranium Registries. It is considered important to establish a European Registry of persons with internal radioactive contamination in the Member countries. The position on autopsy data in the UK and France was established but the establishment of an Internal Dosimetry Registry was an action for the future. Such a Registry would have a similar protocol to that in the USA by seeking donations of organs or whole bodies from occupationally exposed persons; by developing new models based upon these measurements and in vivo and bioassay data; by providing a database of data and models. Kathren (corresponding member in the USA) was keen to collaborate in establishing such a Registry and moves were being considered in the UK, France and FRG to obtain national data. The Registry could also form a focus for the establishment of a common format for all dose records to ease the transfer of workers between countries of the Community. Also intercomparisons of methods of internal dose assessment were vital to proving models and laboratories ability to use them and this would be part of the work of the Committee and ultimately of the Registry.

In addition to the practical problems outlined above, the Committee has established the basis of a working relationship with EULEP, the USA Registries, COGEMA and committees in the UK, France and FRG but would like other contacts in other Member States. It was not possible to include a parallel session on Assessment of Internal Dose at the SRP International Symposium in Malvern in June, 1989.

IV. Objectives for the next reporting period:

To continue work to: develop computer models for excretion analysis; interpret data from air sampling, bioassay and in-vivo monitoring; establish availability of autopsy data; consider compatibility of dose records. Projects are: (i) Preparation of a research proposal on stable isotope metabolic studies for the 1990-1994 EURATOM RP Programme; (ii) Establish the basis of a European Internal Dosimetry Registry; by discussion with EURADOS-CENDOS, EULEP, COGEMA and individual representatives of countries in the European Community. (iii) Consider the implications of the new ICRP lung and GI tract models for dose assessments; (iv) Co-ordinate intercomparisons between the UK and COGEMA; (v) Organise a Workshop on Internal Dose Assessment.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

UKAEA, Harwell
NRPB, Chilton
CEA/IPSN, Fontenay-aux-Roses
GSF, Neuherberg
US DOE, Washington
JAERI, Tokai-mura
Swiss Federal Institute for Reactor Research
CEGB Berkeley
University of Saarland, Homburg (Saar)
GSF, Frankfurt
KfK, Karlsruhe
Hanford Environmental Health Foundation, USA
EULEP Committee on Internal Dosimetry and Standardisation, KfK, Karlsruhe

VI. Publications:

J A B Gibson and R K Bull. Dose Assessment from Bioassay and Body Monitoring Measurements: Practical Experience. Workshop on Biological Assessment of Occupational Exposure to Actinides, Versailles, France, 1988. Rad. Protect. Dosim. (in press).

R L Kathren. The United States Transuranium and Uranium Registries. Workshop on Biological Assessment of Occupational Exposure to Actinides, Versailles, France, 1988. Rad. Protect. Dosim. (in press).

D Nosske, H D Roedler and H Schiefendecker. Fecal Excretion Measurements of the Accidental Plutonium-239 Inhalation - Interpretation by modifying Lung model parameters. Workshop on Biological Assessment of Occupational Exposures to Actinides, Versailles, France, 1988. Rad. Protect. Dosim. (in press).

P Roth, E Werner and Ch Hausen. Application of Stable Isotopes to Assess Biokinetic Data in Humans. Workshop on Biological Assessment of Occupational Exposure to Actinides, Versailles, France, 1988. Rad. Protect. Dosim. (in press).

H Schiefendecker, H Dilger and H Doerfel. Practical experience accumulated at the Karlsruhe Nuclewar Research Centre in detecting incorporated plutonium. Workshop on Biological Assessment of Occupational Exposure to Actinides, Versailles, France, 1988. Rad. Protect. Dosim. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

International Commission on
Radiation Units and Measurements
ICRU
7910 Woodmont Avenue, Suite 1016
USA Bethesda, MD 20814

Contract no.: BI6-A-027-US

Head(s) of research team(s) [name(s) and address(es)]:

Prof. A. Allisy
Intern. Commission on Rad.
Units and Measurements, ICRU
7910 Woodmont Ave, Suite 1016
USA Bethesda, MD 20814

Telephone number: 301-657-26.52

Title of the research contract:

Quantities, units and measurement techniques for ionizing radiation.

List of projects:

1. Quantities, units and measurement techniques for ionizing radiation.

Title of the project no.:

1. Quantities Units and Measurement Techniques for Ionizing Radiation

Head(s) of project:

Professor Andre Allisy

Scientific staff:

I. Objectives of the project:

The development of internationally acceptable recommendations regarding:

(1) Quantities and units of radiation and radioactivity,

(2) Procedures suitable for the measurement and application of these quantities in clinical radiology and radiobiology,

(3) Physical data needed in the application of these procedures, the use of which tends to assure uniformity in reporting.

The ICRU also considers and makes recommendations in the field of radiation protection.

II. Objectives for the reporting period:

Completion of work (publication) of ICRU reports on: (1) use of computers in external beam radiotherapy procedures with high energy photons and electrons, (2) determination of dose equivalents for external radiation sources—part 2, (3) tissue substitutes in radiation dosimetry and measurements, and (4) clinical neutron dosimetry (physics aspects). Completion of drafting work (consideration by the ICRU) of a report on: (1) physical parameters to specify performance of imaging instruments in nuclear medicine.

III. Progress achieved:

During 1988 the International Commission on Radiation Units and Measurements (ICRU) completed work on three reports which are now either published or in press:

(1) ICRU Report 42, Use of Computers in External Beam Radiotherapy Procedures with High-Energy Photons and Electrons (published)
(2) ICRU Report 43, Determination of Dose Equivalents from External Radiation Sources--Part 2 (in press)
(3) ICRU Report 44, Tissue Substitutes in Radiation Dosimetry and Measurements (in press)

ICRU Report 42, Use of Computers in External Beam Radiotherapy Procedures with High Energy Photons and Electrons, continues the series of reports issued by the Commission dealing with dosimetric problems in external beam therapy with high energy photons. The new report covers treatment planning and recording and documentation procedures using computers in external beam therapy. Because of the rapid evolution of computer technology, only the main concepts and basic methods are presented with some specific examples included for illustrative purposes.

ICRU Report 43, Determination of Dose Equivalents from External Radiation Sources--Part II, is the second in a three-report series produced by the ICRU to provide guidance on the practical determination of dose equivalents from external radiation sources. The first of the series, ICRU Report 39, Determination of Dose Equivalents from External Radiation Sources, provided definitions of quantities to be employed in radiological protection monitoring, including the ambient dose equivalent, the directional dose equivalent, the individual dose equivalent penetrating, and the individual dose equivalent superficial. The new report provides the grounds for the Commission's selection of these quantities and the basis for their definition. The third report in this series, which is now in preparation, will provide guidance on the design, calibration and use of instruments required to implement the recommended system of dose determination.

ICRU Report 44 Tissue Substitutes in Radiation Dosimetry and Measurement was prepared in recognition of the fact that the need to measure the absorbed doses within and around the irradiated body tissues necessitates the use of carefully selected materials from which phantoms and radiation detectors can be constructed. The use of such materials

permits determination of absorded doses when information on the energy and nature of the charged particles at the point of entrance is incomplete or fragmentary. To make an appropriate selection of material for such purposes it is necessary to have information on the characteristics of tissue substitutes that affect radiation interaction and the report attempts to provide these. Since the required degree of agreement between the measured "actual" absorbed doses depends upon the intended application of the data, the individual specialties employing tissue substitutes are surveyed in the report including phantom and detector materials used in radiotherapy, radiodiagnosis, radiation protection and radiobiology.

In addition, the printer's manuscript is nowbeing prepared for ICRU Report 45, Clinical Neutron Dosimetry--Part I: Determination of Absorbed Dose in a Patient Treated by External Beams of Fast Neutrons. This is the first of two reports relating to clinical neutron dosimetry.

Work continued during 1988 on the development of reports concerned with (1) absorbed dose standards for photon irradiation and their dissemination, (2) clinical dosimetry for neutrons (dose specification for reporting) (3) fundamental quantities and units, (4) measurement of dose equivalent, (5) statistical methods used in particle counting, (6) stopping power for protons and alpha particles, (7) dose specification for reporting interstitial therapy, (8) performance assessment in digital representation of images, (9) phantoms for therapy, diagnosis and protection and (10) quality assurance in external beam therapy. Being reorganized is ICRU work on chemical dosimetry and specification of imaging instruments in nuclear medicine.

Recently initiated are efforts concerned with (1) stopping power for heavy ions, (2) dose specification for reporting therapy with photons and electrons, (3) calculated photon, electron, proton and neutron interaction data for body tissues, (4) proton therapy, and (5) secondary-electron spectra resulting from charged-particle interactions.

The ICRU is studying the need for new work concerned with (1) hyperthermia and (2) dosimetry at high doses.

IV. Objectives for the next reporting period:

Completion of work (publication) of ICRU reports on: (1) the basis for quantities used in determination of dose equivalent from external radiation sources, (2) tissue substitutes and (3) physics aspects of clinical neutron dosimetry.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

ICRU Report 42, Use of Computers in External Beam Radiotherapy Procedures with High-Energy Photons and Electrons (published)

ICRU Report 43, Determination of Dose Equivalents from External Radiation Sources--Part 2 (in press)

ICRU Report 44, Tissue Substitutes in Radiation Dosimetry and Measurement (in press)

RADIATION PROTECTION PROGRAMME
Progress Report
1988

Contractor:

ICRU - International Commission on
Radiation Units and Measurements
7910 Woodmont Avenue, Suite 1016
USA Bethesda, MD 20814

Contract no.: BI6-A-217-US

Head(s) of research team(s) [name(s) and address(es)]:

Prof. A. Allisy
Intern. Commission on Rad.
Units and Measurements, ICRU
7910 Woodmont Ave, Suite 1016
USA Bethesda, MD 20814

Telephone number: 301-657-26.52

Title of the research contract:

Environmental monitoring needs connected with nuclear reactor accidents.

List of projects:

1. Environmental monitoring needs connected with nuclear reactor accidents.

Title of the project no.:

1. Environmental Monitoring Needs Connected with Nuclear Reactor Accidents

Head(s) of project:

Professor Andre Allisy

Scientific staff:

I. Objectives of the project:

The development of internationally acceptable recommendations regarding the practical aspects of measuring dose equivalent, including environmental monitoring related to nuclear reactor accidents.

II. Objectives for the reporting period:

Completion of the drafting work on an ICRU report on measurement of dose equivalent and revision on the basis of the comments of the report committee.

III. Progress achieved:

The ICRU report committee responsible for the drafting work on an ICRU report on measurement of dose equivalent made substantial progress toward completion of the drafting work aimed at the preparation of a report based on the following outline:

1. Introduction
2. General Considerations
   - Energy dependence
   - Angular dependence
   - Measurement of dose as a function of LET
   - Measurement of kerma (taking into account energy dependence)
   - Adjustment of response of an instrument to approach an ideal one
   - Role of wall thickness and composition on the measurement of the new operational quantities
   - Geometry of detector
   - Role of various types of instruments
3. Characteristics of Instruments
4. Calibration of Instruments
   - Phantoms to be used
   - Reference point
5. Conversion Factors
   (calibration quantities to new operational quantities)
6. Correction Factors
   (sphere to phantom)

All sections have been drafted and subsequently revised and work is now underway on melding these into a cohesive document to enter into the ICRU review process.

IV. Objectives for the next reporting period:

Completion of the drafting work and submission of the draft report to the ICRU for review.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

III B

VERHALTEN UND KONTROLLE DER RADIONUKLIDE IN DER UMWELT

BEHAVIOUR AND CONTROL OF RADIONUCLIDES IN THE ENVIRONMENT

COMPORTEMENT ET CONTROLE DES RADIONUCLEIDES DANS L'ENVIRONNEMENT

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Risø National Laboratory
DK-4000 Roskilde

Contract no.: BI6-B-030-DK

Head(s) of research team(s) [name(s) and address(es)]:

Dr A. Aarkrog
Health Physics Department
RISØ National Laboratory
DK-4000 Roskilde

Telephone number: 02/37.12.12

Title of the research contract:

Behaviour of long-lived radionuclides in terrestrial and marine (North Atlantic Region) environments.

List of projects:

1. Terrestrial environment : Dynamic models of the human foodchain and determination of less wellknown long-lived radionuclides.
2. Marine environment : Experimental studies (turnover of radionuclides in bioindicators), field studies (North Atlantic region - Baltic sea), and Thule studies.

Title of the project no.:

Terrestrial environment a) dynamic models of the human food chain and b) determination of less well-known long-lived radionuclides.

Head(s) of project:

Dr. Sven Poul Nielsen (a), Dr. Elis Holm (b), (Univ. of Lund, Sweden).

Scientific staff:

Mette Øhlenschlæger (a)
Lars Bøtter-Jensen (b)
Qingjiang Chen (b)

I. Objectives of the project:

a) To test available dynamic models, (e.g. NRPB) using the Danish/Nordic fallout data bank, to simplify these models if warranted, to investigate the influence of changes on agricultural practice, which have taken place in the last 25-30 years on the models, and to see if such an influence could be dealt with in future modelling. The perspective of an EEC fallout data bank will also be considered.

b) To develop counting equipment and analytical methods for determining and measuring less well-known long-lived radionuclides in low concentrations in environmental samples.

II. Objectives for the reporting period:

The Chernobyl ^{137}Cs data will be used for similar model investigations as carried out for ^{131}I in 1987. Sensibility and uncertainty analyses will be investigated.

Experiments with an ion exchange method for ^{99}Tc will be carried out for large volume seawater samples. The procedure for Am analysis will be improved in order to avoid contamination from naturally occurring radionuclides.

III. Progress achieved:

a) Dynamic models

Methodology

A computer program, TAMDYN, has been developed for the simulation of environmental transport of contaminants. The program comprises improved numerical techniques for solving the ordinary differential equations of the compartment model using the Runge-Kutta-Fehlberg method and provides facilities for performing sensitivity and uncertainty analyses. The progran includes options to modify the values of the state variables and non-transfer parameters during the solution of the differential equations. The modifications are useful for simulating events like contaminations, harvests etc. The program development was accomplished in connection with a fellowship supported by the IAEA, where Dr. Béla Kanyár was visiting Risø.

This program was used to simultate the ^{137}Cs contamination in Denmark from Chernobyl. The model contains one air, three soil, three plant and four cow compartments, all referring to an area of one square metre. The transport processes of the air-soil-plant system are the traditional ones. An upper soil compartment is introduced to account for resuspension of radioactive material.

The cow compartments reflect two transient and two resident tissues for radiocesium. Because the milk concentration is determined mainly by the blood activity, a simple concentration factor is used to estimate the Cesium content in the milk.

The values of the transfer parameters were derived from the literature. However, these values were modified to get a better fit to the measured data after Chernobyl.

Results

In order to simulate the conditions in Denmark after Chernobyl the input to the air compartment of the model was adjusted to produce air concentrations similar to those observed. The model predictions underestimate the measured values by about a factor 2, which is due mainly to the low deposition values calculated by the model. The main part of the deposition at Risø arrived during rainfa but the measured ground-level air concentrations were too lo to account for the deposition and thereby indicate higher conce trations at higher altitudes.

In order to demonstrate the sensitivity and uncertainty potentials of the model, calculations were made of ^{137}Cs in beef at the predicted maximum level which occurred in the end of May 1986. The sensitivity analysis points out that the potential variation of the maximum beef concentration is caused by two parameters only. The plant interception factor accounts for

45% of the variation, and the conversion factor from muscle to beef accounts for 54%. From the uncertainties assigned to the parameters and keeping the above concentration factor constant, an uncertainty analysis gives a distribution of maximum beef concentrations. Of this variation 80% originates from the plant interception factor and 10% from the washout coefficient.

Discussion

The present model for the transfer of Chernobyl radiocesium in the terrestrial environment near Risø underestimates the observed levels in grass, milk and beef with about a factor 2. The predicted time trends seem to agree reasonably well with the measured during the first year. For the second year the model needs improvement with respect to the resuspension processes. The model needs changes with respect to deposition and resuspension processes. The uncertainty of the predicted maximum beef concentrations is caused mainly by the uncertainty of the plant interception factor.

b) Determination of less well-known long-lived radionuclides

A new method for collecting ^{99}Tc from 200-400 litre sea water by anion-exchange has been developed. Further decontamination of $^{103,106}Ru$ was achieved by repeated evaporation of RuO_4 from 0.1 N H_2SO_4 at 100°C, followed by extraction of TcO_4^- into 5% TIOA-xylene at controlled valence. ^{110m}Ag was precipitated as AgCl. An average overall radiochemical yield of 70% was found for Tc. Decontamination factors were between 3×10^5 and 2×10^7 for Ru and 2×10^5 for Ag. A new method to determine ^{99}Tc in seaweed by wet-ashing and simple extraction into 5% TIOA/xylene was also developed.

The radiochemical procedures for Pu and Am in environmental samples have been scrutinized in order to obtain a more efficient decontamination for natural radioactivity, in particular Th.

A method has been developed for the determination of ^{63}Ni in environmental samples. The samples are ashed and leached with aqua regia whereafter hydroxides are precipitated with ammonia, leaving Ni in the aqueous phase. Nickel is extracted as dimethyl complex by chloroform and backextracted with HCl. Finally, Ni is electroplated onto a copper disc from ammonium sulphate medium at high pH. The radiochemical yield is determined by atomic absorption measurements of stable Ni before and after electrodeposition. The discs are measured beta spectrometry using solid state ion implated detectors and also by windowless anti-coincidence shielded GM gas flow counting. The least detectable activity was 8 and 1 mBq at 3000 min. counting respectively. The method was applied to a series of macro algaes (Fucus vesiculosus) collected at different distances from a nuclear power plant. The ^{63}Ni concentration in the algaes showed a good correlation with distance to the power plant. The relation between ^{63}Ni and ^{60}Co and ^{63}Ni to stable nickel was also investigated.

IV. Objectives for the next reporting period:

The work on the dynamic models in the terrestrial environment will be finalized and reported. The performance of the dynamic models will be compared with that of the equilibrium models.

Steps for decontamination from natural radioactivity (U & Th) will be introduced in the Tc-analysis. A method for producing α-standard sources for spectroscopy will be implemented.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Oak Ridge National laboratory, USA (Dr. F.O. Hoffman)
Studsvik Energiteknik AB, Sweden (Dr. U. Bergström)
National Radiological Protection Board, UK (Dr. J. Brown)
National Research Institute of Radiobiology and Radiohygiene, Hungary (Dr. B. Kanyàr).

VI. Publications:

Chen Qing Jiang, Aarkrog Asker, Dahlgaard Henning, Nielsen Sven P., Jensen H.L., Bruun Jette, Pedersen Anna Holm, Mandrup Karen. Determination of ^{99}Tc in Environmental Samples by Anion Exchange Risø-M-2739 (1988) p. 21.
Chen Qing Jiang, Aarkrog A., Dahlgaard Henning, Nielsen Svend P., Dick Helle, Mandrup Karen. Determination of Technetium-99 in Environmental Samples by Solvent Extraction at Controlled Valence. Risø-M-2671 (1988) p. 31.
Holm E., Aarkrog A., Ballestra S., Lopez J.J. Fallout deposition of Actinides in Monaco and in Denmark following the Chernobyl accident. IV Int. Symp. of Radioecology of Cadarache 14-18 March 1988.
Holm E., Rioseco J., Ballestra S., Walton A.. Radiochemical measurements of ^{99}Tc: Sources and environmental levels. J. of radioanalytical and Nucl. Chem. 123, 167-179 (1988).
Kanyàr, B. and Nielsen, S.P. (1988), Users Guide for the Program TANDYN.
Risø-M-2741.
Nielsen, S.P. and Kanyàr, B. (1988), Simulation of ^{137}Cs contamination in Denmark from Chernobyl. 5th Nordic Radioecology Seminar, Rättvik, Sweden, 22-26 August 1988.

Title of the project no.:

Marine environment: a) Experimental studies (turnover of radionuclides in bioindicators), b) Field studies (North Atlantic region - Baltic Sea), and c) Thule studies.

Head(s) of project:

Dr. Henning Dahlgaard (a), Dr. Asker Aarkrog (b,c).

Scientific staff:

Dr. Sven Poul Nielsen, Dr. Elis Holm, Dr. Heinz Hansen, Dr. Qingjiang Chen.

I. Objectives of the project:

a) To improve knowledge on the turnover of radionuclides in the two most widely used bioindicator organisms in coastal waters: Fucus vesiculosus and Mytilus edulis under field comparable conditions. b) To study the dispersion of waterborne pollution in the northern North Atlantic by means of radioactive tracers discharged from nuclear facilities and to set up models for this dispersion. c) To follow the behaviour of Pu and Am in the benthic environment at Thule, Greenland. The results of this study may be implemented to waste disposal problems.

II. Objectives for the reporting period:

Samples from Mediterranean loss experiment will be taken during 1988, and further laboratory experiments on effect of food is planned. Special emphasis will be put on data processing and publication of accumulated results. The contamination of the North Sea with ^{99}Tc will be investigated. During the Greenland Sea Project in 1988 large volume (400 l) deep water samples will be collected in order to get a better understanding of the vertical mixing in the Arctic waters. The planning of the 1989 expedition to Thule will be carried out.

III. Progress achieved:

a. Experimental studies

The long term in situ Mediterranean Mytilus loss experiment in Monaco (in collaboration with the IAEA laboratory) has been running satisfactorily during all 1988. As compared with the earlier Baltic experiments, the growth rate is much higher. Preliminary results indicate, that in spite of faster growth, higher temperature and higher salinity, the loss rates are not as different from the Baltic data as expected. As a new result, ruthenium-106 shows high retention and long biological half life in Mediterranean Mytilus.

A parallel laboratory experiment in Monaco has shown good agreement with the in situ Mediterranean experiment. In contrary to what was expected, no differences in biological half lives could be detected between two feeding regimes.

The 1985/86 Baltic long term loss experiment in Oskarshamn gave significantly longer biological half lives but also much lover retention for the slow compartments than the earlier Baltic experiment from Forsmark. The reasons for this might be that the two experiments were started at two different seasons. In bioindicator context, the two effects counteracts. However, it indicates one reason for the variability which must be considered in bioindicator monitoring.

Generally, results from the long term loss experiments with Mytilus and Fucus indicate that for most elements studied, the growth rate is apparently more important for the concentration factor and for the bioindicator time integration, than is the actual loss of elements from the slow compartments. This effect is caused by dilution of pollutants present in the bioindicators by new growth.

b) Field studies

During September the group participated in a cruise with the Icelandic research vessel Bjarni Sæmundsson under the international Greenland Sea Project. At 5 stations between Jan Mayen and East Greenland and at 2 stations further south in the Denmark Strait between Iceland and Greenland, depth profiles were sampled with 270 l water samplers. Tc-99, radiocaesium and Sr-90 will be measured. In the same 7 profiles and at the same depths, the physical oceanographers moored automatic recording current meters, that are expected to measure continuously for the next 5 years. Furthermore CTD profiles were taken. The close correlation with the physical oceanographic programme is expected to improve the use of the radioactive tracers considerably.

Almost all samples from the June 1987 Polarstern cruise have been analyzed. It is now clear that the Tc-99 concentration in the North Atlantic region is not at all influenced by the Chernobyl accident. The majority of the technetium signal in East Greenland waters originates from European fuel reprocessing. Surface seawater concentrations were in 1987 measured to 50 - 100 mBq $^{99}Tc\ m^{-3}$.

In co-operation with the German Hydrographic Institute, Hamburg 26 samples of surface seawater were collected in the North and Irish Seas and in the English Channel. The samples were analysed for ^{99}Tc. The highest concentrations were found close to Sellafield and east of Cap de la Hague; The lowest were seen around Cornwall and in the northern part of the North Sea.

The major effect of Chernobyl in the North Atlantic region is the increased radiocaesium level. For the Baltic as a whole, the accident increased the amount of Cs-137 by a factor 20. The total inventory of ^{137}Cs in the Baltic was estimated at 5 PBq. In 1987 the Cs-137 level in the northern part of the Norwegian Sea and in the West Spitzbergen current was doubled and in the Greenland Sea area it varied from a doubling to an increase of a few percent.

c) Thule studies

A number of lichen samples collected in 1984 on Saunders Island, Thule 10 km WNW from the point of impact showed enhanced Pu and Am levels. The radionuclide ratios showed that the activity was from the accident in 1968. Compared with the Pu levels in lichen in 1968, the effective half-life of Pu in the Thule lichens was estimated at 5-10 years. Am-241 apparently had a longer half-life than Pu.

IV. Objectives for the next reporting period:

The last transuranium samples from the Mytilus loss experiment in the Mediterranean will be ready in the autumn. Results of the experiments will be published. Technetium-99 samples from the 1988 Greenland Sea cruise will be analyzed and data will be compared with physical oceanographic measurements. Radiocaesium and Sr-90 will be measured in West greenland waters. Planning of the next Thule expedition will be carried out. It has been necessary to postpone the expedition to 1990 as no ships are available until then.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Manuella Notter, SNV, Sweden
P. Guegueniat, CEA, France
A.D. Bettencourt, L.N.E.T.I., Portugal
C. Nolan and S.W. Fowler
IAEA, Monaco
Gordon Christensen, IFE, Norway
John Smith, Bedford Inst. of Oceanography, Canada
Albert van Weers, ECN Netherlands Energy Research Foundation, Holland
Hartmut Nies, Deutsches Hydrographisches Institut, Hamburg
Elis Holm, University of Lund, Sweden

VI. Publications:

Aarkrog A., Bøtter-Jensen L., Chen Qing Jiang, Dahlgaard H. Hansen, Heinz J.M., Holm Elis, Lauridsen Bente, Nielsen S.P. and Hansen-Søgaard J. Environmental Radioactivity in Denmark in 1986, Risø-R-549 (1988) p. 274.
Aarkrog A. Studies of Chernobyl debris in Denmark. Environmental International Vol. 14, pp. 149-155, 1988.
A. Aarkrog, Carlsson, Chen Q.J., Dahlgaard H., Holm E., Huynh-Ngoc L., Jensen L.H., Nielsen S.P. & Nies H. Origin of technetium-99 and its use as a marine tracer. Nature Vol. 335, No. 6188, pp. 338-340 22 September 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

GREEK ATOMIC ENERGY COMMISSION
N.R.C.P.S. "DEMOKRITOS"
Aghia Paraskevi
GR - 153 10 Attica

Contract no.: BI6-B-293-GR

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Apostolakis
Lab. of Soils and Plant Nutr.
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Dr. E. Papanicolaou
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Greek Atomic Energy Commission
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GR - 153 10 Attica

Telephone number: 00-30-1-6511212

Title of the research contract:

Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region

List of projects:

1. Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region

Title of the project no.: B 16 - B - 293 - GR

Behaviour of long-lived radionuclides in soil-plant systems of the mediterranean region.

Head(s) of project:

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Lab. of Soils and Plant Nutrition
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Dr. E. Papanicolaou
Lab. of Soils and Plant Nutrition
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Scientific staff:

Institute of Biology

A. Nobeli (Ms)

V. Skarlou(Ms)

Institute of Nuclear Technology

J. Bartzis
P. Kritidis
J. Papazoglou

I. Objectives of the project:

The objectives of the project,as approved,refer to the selection of regions in Greece with high degree of contamination,and sampling of the main soil types-in various depths-and of the cultivated or indigenous plants grown on them.Determination of the physicochemical parameters of the soil samples and the radionuclide concentration in the soil and plant samples. Greenhouse experimentation with selected soil types and main agricultural crops to establish uptake rates,and laboratory studies to investigate translocation of radionuclides within undisturbed soil columns. Correlation of climatic,analytical and experimental data and calculation of transfer factors from soil to plants and various products.

II. Objectives for the reporting period:

For the reporting period (May 1st-Dec.31st 1988)the following were met:
1.Selection of regions burdened with a certain degree of contamination
2.Sampling(during two weekly field trips) of soils in two depths for 44 sites and in one depth for 31 sites.
3.Corresponding sampling-wherever possible-of 1, 2, or 3 cultivated plants (wheat,alfalfa, sunflower, tobacco, pepper)
4.Collection,transport and preparation of large quantities of surface soils from 12 sites for the greenhouse experiments
5.Chemical and radio-analysis of soil and plant samples
6.Ordering of materials and supplies for laboratory and greenhouse work

III. Progress achieved

From previous investigations (Antonopoulos-Domis et al.,Papanicolaou,Kritidis) it was determined that certain areas of Greece have been affected by fallout due to the Chernobyl accident in a higher degree than others. It was reasonable that soil sites -preferably cultivated fields- would be selected for sampling within those regions.

1.Methodology

Soil samples were taken in one or two depths(0-20 and 20-50 cm) using a metallic frame of specific dimensions(10 x 10 x 20 cm) for the sampling of the 0-20 cm layer and a standard soil auger for the 20-50 cm depth. On the same sites,plant samples of the cultivated crops were taken on the spot or as close as possible in order to insure similar conditions.

For the presently reported period we collected 88 soil samples(44 sites in two depths) and 31 soil samples in one depth.From all sites, 42 samples of wheat plants,42 samples of alfalfa,9 samples of sunflower,5 samples of tobacco,and 1 sample of a pepper plant were collected.

The samples were transported to the laboratory in plastic bags and were submitted to the usual preparation for analysis.The soil samples were air-dried,ground and passed through a 2 mm sieve.The plant samples were air-dried at 70^{o} C and ground.

The physical and chemical characteristics such as texture,calcium carbonate content, pH , cation exchange capacity and extractable cations were determined in the soil samples.For this purpose,generally accepted methods were used(Methods of Soil Analysis,ASA,Black C.A. Editor in Chief,1965).

The above soil characteristics have been determined in all samples of the first sampling field trip.

Simultaneously the determination of ^{137}Cs ,^{134}Cs and ^{40}K was started and to this purpose the following systems were used.

-HpGe detector with relative efficiency 20-22% with respect to NaI (Tl) 3 x 3 in. crystal and energy resolution 1.9Kev at the 1332.5 peak of ^{60}Co.

-Shielding with 100 mm Pb+2 mm Cu,cylindrical or shielding with 50 mm Pb, rectangular.

-Analyzer "Canbera model 90" with 21504 channels through which analysis of photopeaks is possible,or Analyzer "Canbera model 35 plus" with 4096 channels with the analysis through an on line computer.

-Geometry of cylindrical pot (diameter 73 mm,height 105 mm).

Quantitative determinations were made at the photopeaks of 661.6 KeV for ^{137}Cs , 604.7 and 795.8 KeV for ^{134}Cs and 1460.7 Kev for ^{40}K.

2. Results and Discussion

The data of the physical, chemical and radio-analyses of the soil and plant samples which have been completed so far are under study and no attempt to corellation could render satisfactory conclusions at this stage. The data indicate an existing contamination of the soils with radiocesium ranging from approximately 2 Bq/kg to over 100 Bq/kg and in certain cases contamination of the 20-50 cm layer.

Corresponding plant samples indicate a contamination which in comparison is higher in alfalfa than in wheat (6.9 Bq/kg vs 0.7 Bq/kg) and higher in the wheat straw than in the grain (6.4 Bq/kg vs 0.7 Bq/kg).

For the same period the collaborating in the project group collected agricultural products and soil samples from three of the most productive areas of northern Greece.Correspondingly their results indicate a contamination of the grains of annual crops less than 1 Bq/kg.Their straw content is higher but less than 5 Bq/kg. The content of fruit from perrenial plants (trees) is higher than that of the grains and it is in the range of 2 to 10 Bq/kg. The natural radioactivity due to ^{40}K is one or two orders of magnitude greater than that due to radiocesium contamination.

Bibliography

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(In Press) Presented in the Intern. Conference of Environmental Radioactivity in the Mediterranean Area. Barcelona,Spain, 10-13 May 1988

Papanicolaou E.P. and P.Kritidis. Contamination of the agricultural Land of Greece with radioactive Cesium and its Effect on the growing Crops.
(In Press) Presented in the Intern. Conference of Environmental Radioactivity in the Mediterranean Area. Barcelona,Spain, 10-13 May 1988

IV. Objectives for the next reporting period:

1. Continuation of physical,chemical and radio-analysis of the collected soil and plant samples
2. Selection of new soil sites for soil and plant sampling
3. Establishment and activities related to the greenhouse experiment
4. Laboratory experiment of translocation of radionuclides in undisturbed soil columns
5. Correlation of data for the first stages of the project

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. M. Antonopoulos-Domis
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School of Engineering
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Dr. A. Gatzianas
School of Agriculture
Aristotelian University of
Thessaloniki
Thessaloniki, GR

VI Publications:

Papanicolaou E.P. and P.Kritidis. Present Status of Soil Contamination by long-lived Radioisotopes and current Research Activities Presented, Balcan Scientific Conference on Environmental Protection Varna, Bulgaria,20-23 / 9 / 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Imperial College of Science
and Technology
Exhibition Road
GB- London SW7 2AZ

Contract no.: BI6-B-032-UK

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 0990/23.911

Title of the research contract:

Time-dependent transfer of radionuclides from atmosphere and soil to crops, following simulated reactor accidents.

List of projects:

1. Time-dependent transfer of radionuclides from atmosphere and soil to crops, following simulated reactor accidents.

Title of the project no.:

Time-dependent transfer of radionuclides from atmosphere and soil to crops, following simulated reactor accidents.
B16-032-UK

Head(s) of project:

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Silwood Park, Buckhurst Road
Ascot, Berkshire, SL5 7TE, U.K.

Scientific staff:

Mrs. E. Goodyear (Grade 3 technician)
Mr. W.R.C. Munro (Research Student)

I. Objectives of the project:

To further the understanding of the factors affecting uptake and subsequent retention of radioactive aerosols on aerial plant parts, and elucidate the relative importance of soil-plant and air-plant pathways for selected radionuclides on the ultimate collective dose to man. To determine the effect of temporal changes in the availability of radionuclides in the soil on uptake into crops.

II. Objectives for the reporting period:

Continuation of the lysimeter studies, using crop rotation with winter oilseed rape, winter wheat, and cabbage. Counting of backlog of samples from 1987 experiments. Dual-isotope studies on the relative importance of soil and air to plant pathways for contamination of edible crop parts at different growth stages.

III. Progress achieved:

1. Methodology

During the reporting period an experiment was performed using dual isotopes to separate out contamination with Cs of aerial plant parts by direct deposition from the atmosphere and uptake via the roots, respectively. The experimental crop was oil-seed rape, grown in a loam into which was mixed ^{137}Cs, after contamination with ^{133}Cs-labelled 5μ silica aerosol in the wind-tunnel under defined aerodynamic conditions, with a wind-speed of 2.0 cm s^{-1}. The plants were then transferred to the field, either exposed or placed under a transparent roof to exclude rain. They were grown on for a period of 85 days, at which a final harvest was taken, with intermediate harvests at 22 and 51 days, as well as an initial harvest immediately after contamination. Soil cores were also taken at each harvest. All plant and soil material was counted by gamma spectrometry for ^{137}Cs and by the same technique, following neutron activation analysis, for the ^{133}Cs. In addition, measurements of ^{133}Cs deposited onto the soil surface beneath the crop in the wind-tunnel were made at the time of application. Velocities of deposition to crop and soil were calculated, together with transfer factors for ^{137}Cs uptake and loss of ^{133}Cs from the aerial parts at each harvest.

The lysimeter studies were continued by analysing the backlog of samples from 1987 and calculating relevant transfer factors for winter wheat, cabbage, and oil-seed rape. An attempt was made to grow winter wheat from 1987 to 1988 on the lysimeters, but this failed due to inclement weather conditions, notably excessive rainfall. In March/April 1988 the lysimeters were sown with oil-seed rape and spring wheat, which were harvested and counted on 3 separate occasions, up to maturity (in the case of wheat). Unfortunately the suppliers of oil-seed rape accidentally supplied a winter cultivar and, consequently, this resulted in the plants remaining in a vegetative condition, so that it was not possible to analyse the Cs content of their seeds. For winter 1988/9 the lysimeters have been planted up with cabbage.

2. Results

Table 1 - Soil-to-plant transfer factors for Savoy cabbage grown on Eutric gleysol (Bq per g dry plant/Bq per g dry soil)

		Cs 134		Cs 137	
		ploughed	minimal tillage	ploughed	minimal tillage
Harvest 1	stem	1.15e-3	5.19e-3	2.14e-3	2.14e-3
(43d)	leaf	2.06e-3	5.19e-3	2.71e-3	2.66e-3
Harvest 2	stem	4.02e-4	1.97e-3	9.63e-4	1.01e-3
(62d)	inner leaf	4.14e-4	1.81e-3	1.05e-3	8.96e-4
	outer leaf	4.60e-4	2.59e-3	1.49e-3	1.59e-3
Harvest 3	stem	2.31e-4	1.04e-3	5.35e-4	4.03e-4
(134d)	inner leaf	2.87e-4	1.05e-3	6.42e-4	5.60e-4
	outer leaf	4.72e-4	2.49e-3	1.07e-3	6.72e-4
Harvest 4	stem	1.90e-4	1.14e-3	4.68e-4	1.57e-3
(188d)	inner leaf	1.63e-4	6.93e-4	4.65e-4	1.04e-3
	outer leaf	7.51e-4	3.24e-3	2.01e-3	4.17e-3

Table 1 shows the results for the 1987 cabbage grown on eutric gleysol, with both ploughing and minimal tillage and cultivation. Over the course of the four harvests, the transfer factors did not show again clear overall trends. The most notable feature is the contrasting behaviour of the Cs, according to its duration in the soil (5 years for ^{137}Cs and 1 year for ^{134}Cs) for the two cultivation types. Overall, in ploughed soil the transfer of the "older" Cs was greater than that applied recently, whereas with minimal tillage the opposite situation prevailed. A generally similar pattern was observed for the other soil types.

In the wind-tunnel experiment, velocities of deposition were calculated of 0.18 ± 0.05 cm s^{-1} and 0.16 ± 0.03 cm s^{-1} for the plants and soil, respectively. After transfer to the field the foliar ^{133}Cs was lost at an exponential rate, this being considerably greater when the plants were exposed to rain ($T_{\frac{1}{2}}$ = 13d cf. 20d when covered) (Fig. 1). Soil to plant transfer factors showed a small, but consistent, increase in covered compared with exposed plants, but both declined overall with time (Fig. 2).

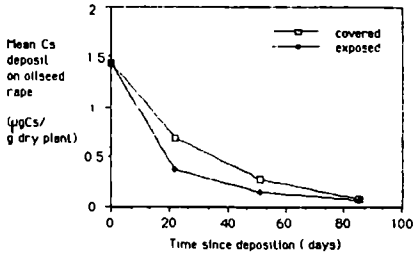


Figure 1 - Field loss of Cs labelled aerosol from oilseed rape under covered and exposed conditions

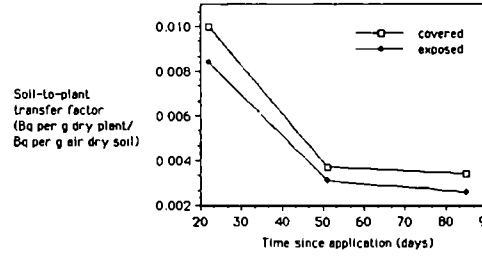


Fig 2 - Soil-to-plant transfer factors for oilseed rape under covered and exposed conditions

3. Discussion

The lysimeter experiments have yielded unexpected results in that Cs did not show any major fall in availability over a 4 year period and, indeed when the soils were ploughed resulted in a higher availability. This suggests that soil processes, possibly connected with microbial activity or water retention, may have a major influence on Cs availability which has important implications for crop contamination in the years subsequent to accidental deposition. The wind-tunnel experiment points towards climatic influences having important effects on the retention of Cs which has contaminated aerial parts of a crop by root uptake, as well as by direct deposition where wash-off might be anticipated.

IV. Objectives for the next reporting period:

Continuation of lysimeter studies with cabbage, with sequential harvests up to maturity. Estimation of changes in the exchangeable: total Cs ratio in experimental soils from all harvests throughout the programme. Measurement of contribution of soil resuspension to contamination of aerial plant parts, using Ti as a tracer. Dual-isotope experiment with cabbage in the wind-tunnel, followed by assessment of the fate of the foliar and soil applied Cs up to maturity.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None

VI. Publications:

None

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-B-033-D

Lehrgebiet Strahlenschutz in der
Kerntechnik der Rhein.Westfälissch.
Technischen Hochschule Aachen
Templergraben 55
D-5100 Aachen

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. H. Bonka
Lehrgebiet Strahlenschutz in der
Kerntechnik der RWTH Aachen
Templergraben 55
D-5100 Aachen

Telephone number: 0241/80.54.40

Title of the research contract:

Improvement of models for the calculation of the dry deposit of radionuclides and radioiodine bound to aerosol particles.

List of projects:

1. Measurement of the influence of the height on the diffusion coefficients of the turbulence both in the field and in a windtunnel.

Title of the project no.: 1

Measurement of the influence of the height on the diffusion coefficients of the turbulence both in the field and in a wind tunnel.

Head(s) of project:

Prof. Dr. rer. nat. H. Bonka

Scientific staff:

Dr.-Ing. H.-G. Horn
Dipl.-Ing. R. Kreh

I. Objectives of the project:

- Measurement of the vertical distribution of eddy diffusion-coefficient in and above different kinds of vegetation (field and wind tunnel experiments)
- Use of the results of the measurements to improve the models for the calculation of the dry deposition of radionuklides bound to aerosol particles and radioiodine.

II. Objectives for the reporting period:

- Measurements (wind tunnel and field) with three dimensional probes.
- Calculation of the diffusion coefficients from the measurements.
- Recalculation of the dry deposition on grass with the measured turbulent flow.

III. Progress achieved:

1. Methodology :

- Measurement of velocity profiles in sparcely wooded areas
- Windtunnel experiments on the deposition velocity on model grass
- Development of a "Random Walk" computer code for the combined calculation of atmospheric dispersion and dry deposition.

2. Results :

Sparcely wooded areas are often found in agriculturally used fields in Central Europe. In order to calculate the deposition velocities for these structures, measurements of the wind velocity profile have been continued. The measurement results were reducted with statistical data and can be used in the deposition velocity programs. Fig. 1 shows a typical result of the measurements in sparcely wooded areas (approximated by splines).

Deposition experiments have been carried out using the model grass that has been constructed and measured in the wind-tunnel . The expected typical curve of the deposition velocity results for particles of different sizes. First measurements of the deposition velocity were found to be of higher magnitude. The examination of the stirring influences is going on.

Up to now computer codes for calculation of dry deposition base on a one-dimensional vertical transport model in and just above vegetation. In order to obtain a better combination of atmospheric dispersion and dry deposition, a computer code has been developed using eddy flow parameters directly. That means, they can be calculated without the eddy diffusion coefficients. This program was tested using simulated eddy flow parameters and led to reasonable results. An example of these calculations is shown in Fig. 2 .

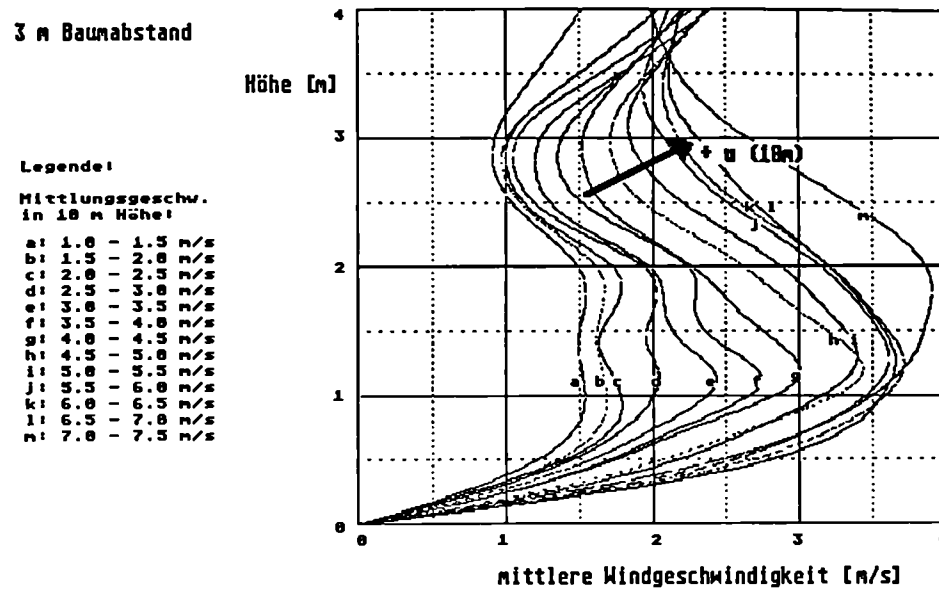


Fig. 1. : Measurements of the wind velocity approximated by a spline , in a distance of 3 m to a tree , for different mean wind velocities in a heigth of 10 m.

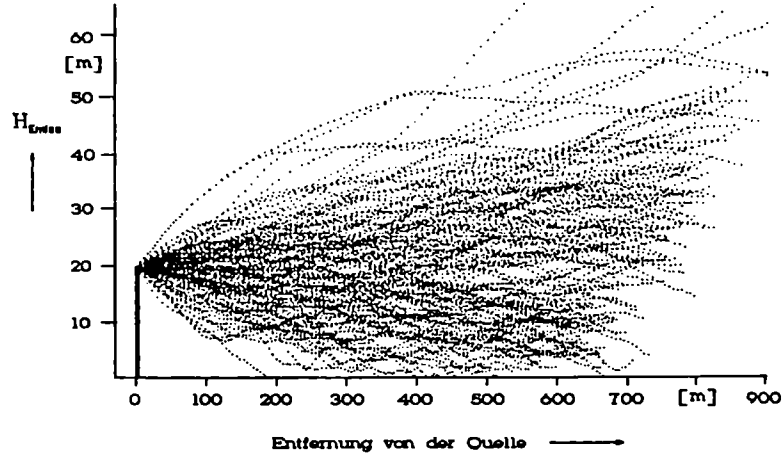


Fig. 2. : Simulated calculation with $\bar{u} = 2\,\frac{m}{s}$, $\Delta t = 3\,s$
Number of steps : 300 , Number of particles : 150

The three dimensional measurements of the wind velocity unexpectedly turned out to be very difficult. Just before completing the calibration measurements, the probe broke down. therefore the total calibration measurements had to be repeated after the probe was repaired. We haven't yet finished to permutate the new calibration measurements into the neccessary coefficient set ups. Therefore measurements with the 3-d-probe can only be carried out in 1989.

3. Discussion :

Although the deposition experiments in the wind-tunnel have been carried out very precisely, the results can differ to field experiments. Therefore our attention has to be directed on limiting conditions influencing the flow as well as possibly neccessary correction faktors.

IV. Objectives for the next reporting period:

- **Measurements with three-dimensional probes (windtunnel and field)**
- **Measurements of dry deposition in the windtunnel with natural grass in comparison with model grass.**
- **Fitting the measurement results of the three-dimensional probe to the one-dimensional "random walk" program and comparison with the measurement results in the windtunnel and field.**

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

BRENK Systemplanung
Heinrichsallee 38
D-5100 Aachen

Contract no.: BI6-B-055-D

Head(s) of research team(s) [name(s) and address(es)]:

Dr. Ing. H.D. Brenk
BRENK Systemplanung
Heinrichsallee 38
D-5100 Aachen

Telephone number: 0241/51.33.21

Title of the research contract:

Modelling of the deposition and postdeposition behaviour of accidentally released radionuclides in urban and suburban environments.

List of projects:

1. Modelling of the deposition and postdeposition behaviour of accidentally released radionuclides in urban and suburban environments.

Title of the project no.:

Head(s) of project:

Dr.-Ing. H.D. Brenk
BRENK SYSTEMPLANUNG
Heinrichsallee 38
D-5100 Aachen

Scientific staff:

Dr. rer.nat. H. de Witt — BRENK SYSTEMPLANUNG
Dr. rer.nat. R. Görtz — Heinrichsallee 38
A.G. Knaup — D-5100 Aachen

I. Objectives of the project:

1. Deposition to urban surfaces
   a) Dry deposition
   b) Wet deposition
   c) Resuspension
2. Postdeposition radionuclide behaviour on urban surfaces
   a) Hydrologically induced transport processes
   b) Hydrological characteristics of urban surfaces
3. Physicochemical radionuclide behaviour on surfaces
4. Decontamination experiments on urban surfaces
   a) Natural decontamination
   b) Forced decontamination

II. Objectives for the reporting period:

Reporting period: 01.01. - 31.12.1988

2. Postdeposition radionuclide behaviour on urban surfaces
   a) Hydrologically induced transport processes
   b) Hydrological characteristics of urban surfaces
3. Physicochemical radionuclide behaviour on surfaces
4. Decontamination experiments on urban surfaces
   a) Natural decontamination
   b) Forced decontamination

1. Methodology

The research work proposed is intended to explore the literature and other relevant sources for pertinent information related to the deposition and post-deposition radionuclide behaviour on urban surfaces including the physicochemical radionuclide behaviour on urban surfaces and decontamination experiments on such surfaces.

2. Results

The transport of surface deposits, e.g. radioactive or non-radioactive substances, associated with the movement of water is a very complex phenomenon and may be divided into washoff and migration. Washoff refers to the hydrologically induced transport of a substance associated with the erosive action of surface runoff while migration denotes the movement of a substance through the soil surface into the upper soil zone with infiltrating water.

Surface runoff (or overland flow) is defined as the excess of precipitation that does not remain on the area receiving the rainfall. The understanding of the formation of surface runoff requires knowledge of the state of the physical parameters governing the flow of water over and through media of the drainage system as well as the climatic and physiographic factors involved.

The principal mechanisms causing a material to become immobilized on a surface includes a number of physical and chemical processes incl. adhesion, absorption as well as chemical reaction. Type and extent to which these processes contribute to the adhesive strength is difficult to predict and depends on the state and complexity of the interacting surface-pollutant system.

Considering all these results and experiences, decontamination experiments on some urban surfaces have been constructed. On such complex surfaces it can be shown that ammonium nitrate is the most efficient decontamination medium except on clay roof tiles due to its high affinity to sorption processes of Cs to clay minerals.

3. Discussions

The relevant literature related to the hydrologically induced transport of nonpoint source pollutants indicates that a full understanding of the problem requires the following basic information, cf. DONIGIAN et a. (54):

1. Characterization of the hydrology of the drainage area,
2. Characterization of the water induced transport,
3. Type and extent of physico-chemical interactions of surface deposits on a receptor surface.

Not all of these aspects are well understood. Consequently, the accuracy of a model is limited by various factors incl. how well the individual processes involved can be quantitatively described. Each of these processes will be discussed below.

The quantity of a radionuclide remaining on a surface depends on a variety of factors incl. the initial mass load, the manner of radionuclide deposition (dry and wet), the time and type of decontamination, the type of surface, the rate of drying a liquid, and the physicochemical character of the deposits. The wet-decontamination-method related efficiency data of urban surfaces, i.e. asphalt, concrete, roofs, available for this study from BAKER et al. (72), MILLER (73), OWEN (74), MALONEY et al. (75) etc. provide the following estimates of the fraction remaining:

Dry deposited particulates: 30 - 50 %
Wet deposited particulates: 70 - 90 %.

The data are specific for low surface loads and small particle deposits. The data indicate, that a major fraction of wet and dry deposited radionuclides on impervious solid surfaces may not be readily available for transport by surface runoff. The wet deposition related data are in agreement with recent experimental data of the RISO National Laboratory, where a minor fraction (appr. 10 %) of the fallout activity in precipitation has been found in the runoff water of an experimental roof covered with numerous roofing materials, e.g. cement, tiles, coated and uncoated eternite (76).

The results of the decontamination experiments lead to the hypothesis that not physical properties like porosity are important parameters but chemical interactions of Cs-ions with the grid-structure of the material. Subsequently the possible interpretations of the fact that clay is hard to decontaminate is, that clay as an end-product of weathering processes fix especially large kation as e.g. Cs^+ so strong that only a few ions are available for ion-exchange processes like $Cs^+ \rightleftharpoons NH_4^+$. These hypothesis has to be proved by further research work.

54 DONIGIAN, A.S. et al. in: Modeling of Rivers (ed. by SHEN, M.W.), Chapter 12, J. Wiley and Sons, New York 1979
72 BAKER, T.P. et al., AFSWC-TN-58-3, May 1958
73 MILLER, C.F., U.S. Naval Radiol. Defense Lab., San Francisco CA, March 1958 (CEX 57.1)
74 OWEN, W.L., USNRDL-TR-277, Nov. 1958
75 MALONEY, J.C. et al., NDL-TR-66, May 1966
76 ROED, J., Personal Communication, June 1984

IV. Objectives for the next reporting period:

1. Deposition to urban surfaces
   a) Dry deposition
   b) Wet deposition
   c) Resuspension
2. Postdeposition radionuclide behaviour on urban surfaces
   a) Hydrologically induced transport processes
   b) Hydrological characteristics of urban surfaces
3. Physicochemical radionuclide behaviour on surfaces
4. Decontamination experiments on urban surfaces
   a) Natural decontamination
   b) Forced decontamination

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Associated Nuclear Services
Eastleigh House
60 East Street
GB- Epsom, Surrey KT17 1HA

Contract no.: BI6-B-194-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. P.J. Coughtrey
Biological Sciences Group
Associated Nuclear Services
Eastleigh House, 60 East Street
GB- Epsom, Surrey KT17 1HA

Telephone number: 40531

Title of the research contract:

Experimental programme to support the development of dynamic models describing carbon-14 (and other B-emitters) in soil-plant-animal systems.

List of projects:

1. Experimental programme to support the development of dynamic models describing carbon-14 (and other B-emitters) in soil-plant-animal systems.

Title of the project no.:

Experimental programme to support the development of dynamic models describing carbon-14 and other β emitters in soil-plant-animal systems.

Head(s) of project:

Dr. P.J. Coughtrey.

Scientific staff:

J.A. Kirton.
N.G. Mitchell.
C.J. Beetham.

I. Objectives of the project:

The overall objective of the project is to improve knowledge on the behaviour of low energy β emitters, particularly C-14, in soil-plant-animal systems in order to provide a basis for models used in radiological assessments.

II. Objectives for the reporting period:

(i) To continue to develop, test and validate techniques and facilities for extraction of C-14 from labelled plant and soil materials and to test and operate laboratory facilities for investigations on transfer of C-14 from soil to vegetation.

(ii) To use these techniques and facilities to investigate C-14 transfer to plants via roots in controlled conditions.

(iii) To derive rate coefficients for use in the soil-plant-animal model, SPADE, developed by ANS for MAFF.

(iv) To undertake preliminary sampling in field conditions to provide data for model validation.

III. Progress achieved:

1. METHODOLOGY

The solution culture apparatus described in previous progress reports was further modified and tested and a further experiment was attempted to provide assurance that observed transfers were due to root uptake of C-14.

The soil-exposure system described in previous progress reports was used for a further experiment with *Lolium perenne*. This allowed for destructive harvests at 9.4 and 29.6 h, and 27.25 d after administration of C-14 to soil as bicarbonate. In addition to sampling of roots, bases, shoots and soil at each harvest, the ventilation stream passing over control and contaminated pots was sampled frequently to monitor losses of C-14 from soil as $^{14}CO_2$.

2. RESULTS

Analysis of samples from the most recent culture solution experiment indicates that 0.29-1.0% of administered C-14 was present in plants at 48 h post-administration. Losses of administered C-14 from solution as a result of aeration ranged from 25 to 70% of the original. 61-84% of C-14 in plants was present in shoots rather than roots. Concentration ratios (Bq g^{-1} fw in plant/Bq ml^{-1} solution) ranged from 23 to 78 with a mean of 61 when estimated on the basis of initial solution concentrations or 65 to 292 with a mean of 184 when estimated on final solution concentrations. 1.6 to 9.7% of administered C-14 was detected in the ventilation stream passing through the top chamber surrounding the plant shoots.

Analysis of results from the most recent soil experiment indicates that ~36% of administered C-14 was lost from soil surface by 9.4 h post-administration, rising to 91% at 27.25 d post-administration. Only 0.02% of administered C-14 was detected throughout the experiment in air passing over soil of the uncontaminated pots. Total C-14 in plants increased from 9.4 to 29.6 h post-administration and then declined to 27.5 d post-administration. At all three times shoots accounted for the majority of C-14 present in the plants. Mean concentration ratios for plant shoots (Bq g^{-1} fw shoot/Bq g^{-1} fw soil) were 0.24 at 9.5 h, 0.51 at 29.6 h and 4.3 at 27.25 d post-administration.

Results of preliminary field sampling of native plant species near a nuclear installation confirmed that C-14 was detectable above natural background. This was most marked for *Juncus effusus* (a rush) and

least marked for *Polytrichum commune* (a moss). Samples with the highest specific activity were those with the highest stable carbon content.

3. DISCUSSION

The solution culture experiments, in conjunction with existing literature provide further evidence for the uptake of C-14 present in solution as bicarbonate by roots of higher plants such as *L.perenne*. No attempt has been made to investigate the underlying physiological mechanism for this uptake. Uptakes observed in culture solution experiments were confirmed by the results of controlled experiments using plants grown in soil. In both cases, losses from the system would have been detected only if C-14 was present as $^{14}CO_2$. It would clearly be of value to repeat the present experiments investigating losses as other gases or vapours (e.g. ethylene) to provide an insight into the underlying mechanisms.

Though the uptakes observed appear to be relatively small when based on plant contents at a single point in time, it is important to recognise that, once transferred from soil to plant, C-14 will be lost rapidly by respiration processes or retranslocated to roots as photosynthetate. The results of the present experiments can not be explained using simple specific activity models even assuming that the observed uptakes are significant underestimates. This reflects the lack of equilibrium in single administration experiments. A similar situation could be expected in field conditions. This is particularly relevant when atmospheric C-14 concentrations are declining and previously incorporated C-14 in soil provides a source for plant uptake. In these circumstances the specific activity of plant will represent a combination of atmospheric and soil conditions.

In field investigations of C-14 around nuclear installations several authors have attributed variations in C-14 specific activity of various samples simply to differences in their growth and development relative to atmospheric C-14 levels. The results of the present experiments in conjunction with preliminary field sampling indicate that uptake from soil is a factor worthy of consideration in field and modelling investigations.

IV. Objectives for the next reporting period:

The project completes on 31 March 1989. Items worthy of further consideration include:

(i) Repeated solution culture and soil experiments using a range of plant species and soil types over a variety of timescales.

(ii) More intensive field sampling with particular attention to soils with a high proportion of aerenchyma tissue (e.g. *J.effusus*).

(iii) Development of time-dependent mathematical models which can allow for root uptake of C-14 as well as photosynthetic fixation of C-14.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

The work reported here has been undertaken in collaboration with the Department of Botany, University of Bristol, Woodland Road, Bristol, BS8 1UG (Dr. M.H. Martin) using facilities at the Department of Chemistry, University of Surrey, Guildford, GU2 5XH. Analysis of environmental samples has been undertaken by the United Kingdom Atomic Energy Authority, Harwell (Ms. J. Walker). The work has been supported by the UK Ministry of Agriculture, Fisheries and Food, Food Science Division.

VI. Publications:

A draft final report on the project has been submitted. It is anticipated that this will form the basis for further scientific publication(s) once amendments have been received and the final version has been issued.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Katholieke Universiteit Leuven
KUL
Naamsestraat 22
B-3000 Leuven

Contract no.: BI6-B-035-B

Head(s) of research team(s) [name(s) and address(es)]:

Prof A. Cremers
Centrum voor Oppervlaktescheikunde
en Colloïdale Scheikunde, K.U.L.
Kardinaal Mercierlaan 92
B-3030 Leuven (Heverlee)

Telephone number: 016/22.09.31

Title of the research contract:

Dynamics of radionuclide chemistry in soils and sediments.

List of projects:

1. Dynamics of radionuclide chemistry in soils and sediments.

Title of the project no.:

Dynamics of radionuclide chemistry in soils and sediments

Head(s) of project:

Prof.A.CREMERS

Scientific staff:

A.Maes, J.De Brabandere, F.Van Elewijck, P.De Preter, J.Tits

I. Objectives of the project:

The main objective of the project is a physico-chemical and mechanistic study of the geochemical behaviour of radionuclides in natural systems such as soils and sediments. The chief emphasis of the work is on the geochemical phase associations of radionuclides and dynamic aspects of radionuclide interception and remobilization processes. The various factors which are being considered are the geochemical characterization, physico-chemical conditions and microbiological effects.

II. Objectives for the reporting period:

The main activities for the 1988 period were directed towards the following objectives:

- the interpretative study of cesium retention in soils and sediments
- the humic acid chemistry of technetium and technetium aging in soils
- geochemical phase association of Zn-65 in soils
- humic acid chemistry of Europium

III. Progress achieved:

RADIOCESIUM IN SOILS AND SEDIMENTS

Considerable progress has been made in the identification of the key factors which regulate the sorption-desorption of radiocesium in soils and sediments. Such understanding is necessary for the assessment of the radiological impact of radionuclide discharges in the environment.

Methodology

The procedure allows to characterize the number and ion selectivity pattern of the sites, highly specific for poorly hydrated alkali cations and is based upon the use of the silver-thiourea complex (AgTU) as a masking agent for the low selectivity exchange sites in clay minerals and organic matter. In the early stages, the capacity of the high selectivity pool- located in interlayer zones at the illite clay particle edges - and the ion selectivity pattern were determined by separate sets of cesium sorption experiments. More recently, a procedure has been developed for obtaining the product of both properties in a single experiment. The quantitative relation has the form:

$$K_D(Cs) = \frac{[HAS]\ K_c(Cs/K)}{m_K} \qquad (1)$$

in which $K_D(Cs)$ refers to the K_D value of radiocesium, [HAS] the number of high affinity sites and $K_c(Cs/K)$ the Cs/K selectivity coefficient in the HAS. Eqn(1) expresses the fact that $K_D(Cs)$ mirrors the solid/liquid distribution coefficient of K (between HAS and solution), amplified by a characteristic selectivity factor. The combined parameter [HAS]Kc(Cs/K) is obtained by measuring the $K_D(Cs)m_K$ value as a function of m_K, in the presence of AgTU(.015M) (results shown below). The competitive effect of other ions, such as NH_4, as compared to K, can be obtained on the same basis, using the eqn.

$$K_D(Cs) = \frac{[HAS]\ K_c(Cs/NH_4)}{m_{NH4}} \qquad (2)$$

It is readily obvious that the ratio of $tsK_D(Cs)m_K/K_D(Cs)m_{NH4}$ for a given substrate directly yields the $K_c(NH_4/K)$ value in the HAS. Furthermore, it can readily be shown that the effect of introducing NH_4-ions on $K_D(Cs)$ is given by the eqn

$$\frac{K_D^{K}(Cs)}{K_D^{K+NH4}(Cs)} = 1 + K_c(NH_4/K)\frac{m_{NH4}}{m_K} \qquad (3)$$

in which $K_D^{K}(Cs)$ refers to K_D of radiocesium at some given K concentration and $K_D^{K+NH4}(Cs)$, the value in the mixed condition (keeping m_K constant).

Results and discussions

The procedure described was applied to soils and marine sediments, covering a representative textural range. The number of HAS varies from about .02 (sandy) to 0.2 meq/kg (clay) in soils. For marine sediments, the upper limit of HAS amounts to 0.6 meq/kg. These limits correspond to a range of 0.2 to 7 Curies/kg(Cs-137). The HAS are exclusively associated with the mineral substrate component and this appears to be the same for the various substrates studied so far (illite clays, geological clays, soils, marine sediments) as indicated by a common value for the Cs/K selectivity coefficient ($2\text{-}3.10^4$). Such conclusion is further corroborated by the finding that, for the various substrates studied, a common value of about 6 is found for $K_c(NH_4/K)$. This is illustrated in fig.1 for the particular case of a loamy soil. This figure shows that, at low K concentration, $K_D(Cs)m_K$ is not a constant indicating that the masking procedure is in effect based on a difference in selectivity of K and AgTU for the HAS. At high K (or NH_4) levels, corresponding to a K (or NH_4)saturation in the HAS, the $K_D(Cs)m_K$ or $K_D(Cs)m_{NH4}$ plateaus directly yield the $[HAS]K_c(Cs/K)$ or $[HAS]K_c(Cs/NH_4)$ values.

Referring to eqn(3), this is a rather important finding in that it clarifies the role played by NH_4-ions in the radiocesium sorption-desorption process in geochemical substrates. Eqn (3) was tested for 12 marine sediments from the Loire estuary (from very sandy to clay textures). Introducing NH_4 at $1.5.10^{-3}$M (as compared to 10^{-2}M for K in sea water) leads to a decrease in $K_D(Cs)$ by a factor of 1.95(±.19) in agreement with predictions based on eqn(3).

The highly competitive effect of NH_4-ions in the specific sites has some very important implications for the fate of radiocesium in variuous compartments of the biosphere. First of all, it may explain the remobilization of radiocesium in freshwater lakes from anoxic sediments in which high interstitial levels of NH_4 are generated. Secondly, a direct link can be expected between eutrophication and radiocesium mobility in surface waters. Such effects can be expected in river sediments where, as a result of urban and industrial discharges, high levels of NH_4 may be found. Thirdly, the high selectivity of NH_4 for the specific sites identifies an important factor in the soil-plant transfer process of radiocesium. In particular, it may explain some of the higher transfer factors of radiocesium in field conditions (often characterized by NH_4 levels exceeding those of K) as compared to greenhouse conditions. This particular effect is now under study for Cumbrian peat soils which are nearly permanently water logged, a condition leading to high interstitial NH_4levels. Finally, this NH_4 effect may explain some of the high radiocesium transfer factors in so-called bio-indicator plants; such high Cs-accumulation may be related to substrate conditions rather than organism specific.

TECHNETIUM IN SOILS

The problem of the Tc distribution between high and low molecular weight humics and the influence of alternating oxic and reducing conditions was further studied in 1988. Ligand exchange between Tc-humic and Tc-fulvic acid complexes is a fast process in suspension but very slow under field conditions in podzol soils. The affinity for Tc is similar for humic and fulvic acids from podzol soil. The low molecular weight fraction of Boom clay has the highest affinity. Reoxidation of Tc-humic and Tc-fulvic acid complexes is extremely slow under field conditions in podzol soil, in contrast to the reoxidation kinetics in suspension.

THE BEHAVIOUR OF ZN IN SOILS AND SEDIMENTS.

The theoretical multi-site model of adsorption which was used to describe the Zn adsorption versus pH in the pure subphases oxide and humic acid and their mixtures was further tested in an integral soil system (loamy soil from Meerdaal,Belgium). In such systems, ion exchange adsorption must be incorporated into the model, in addition to adsorption on oxides and humic acids. The influence of ion exchange is important below pH 5 in which range specific adsorption in oxides and the complexation in humic acids are less important. The adapted model however still does not fully describe the experimental Zn adsorption edge

HUMIC ACID CHEMISTRY OF EUROPIUM.

The ion exchange procedure developed for measuring the stability constant of Europium-humic acid complexes has been further developed and given a rigourous thermodynamic basis taking into account ionic strength effects. The procedure has also been applied to americium humic acid complexes; the stability of the Am-HA complex is practically identical to the one for Eu-HA.

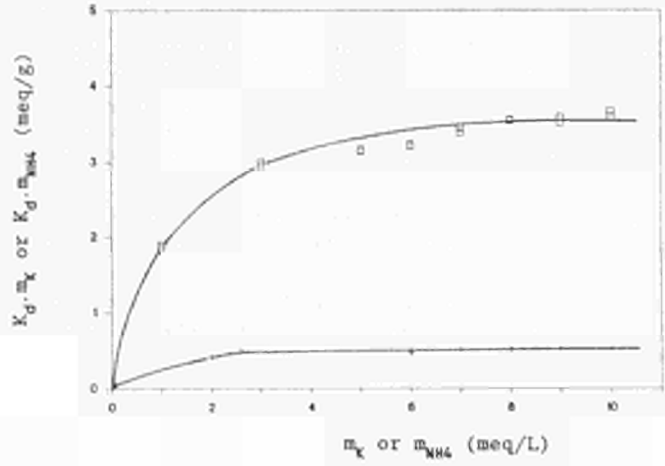


Fig.1. $K_D.m_K$ and $K_D.m_{NH4}$ plot versus m_K(or m_{NH4}): Plateau values are 3.54(K) and .52(NH_4)meq/g. The ratio, i.e. $K_c(NH_4/K)$ is 6.8.

IV. Objectives for the next reporting period:

1. Radiocesium in soils and sediment
   -Development of routineprocedures for characterizing the interception potential of freshwater sediments
   -Application of methods developed to soils representative for natural ecosystems such as peat soil:s (Cumbria)
2. Retention mechanistics of radiostrontium in soils and sediments
3. Retention mechanistics of Zn-65 in soils and sediments

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

-Laboratoire de Biochimie et Radiochimie (J.Pieri),Université de Nantes, France

-Harwell Laboratory, Env.& Medical Sciences Div. (J.Sandalls),Harwell, U.K.

VI. Publications:

1. A.Cremers, A.Elsen, and A.Maes. Solid liquid distribution of radiocesium and radiostrontium under in situ conditions (Abstract) IV. Int.Symp.Radioecology, Cadarache, 1988, p D-67.
2. A.Cremers, A.Elsen, P.De Preter and A.Maes. Quantitative analysis of radiocesium retention in soils. Nature 335, 247-249,1988.
3. A.Maes, J.De Brabandere and A.Cremers. A modified Schubert Method for the measurement of the stability of Europium Humic acid complexes in alkaline conditions. Radiochimica Acta 44/45,51-57(1988).
4. A.Cremers, J.Vancluysen, A.Elsen, A.Maes. Radiocaesium interception in estuarine sediments: a quantitative interpretation. Nature, submitted 1988
5. A.Cremers, P.De Preter, S.Luyten and L.Van Loon. Remobilization of radiocaesium from anoxic sediments: an ion exchange process. Nature, submitted 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Nuclear Energy Board
3 Clonskeagh Square
Clonskeagh Road
IRL- Dublin 14

Contract no.: BI6-B-218-IRL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.D. Cunningham
Nuclear Energy Board
3 Clonskeagh Square
Clonskeagh Road
IRL- Dublin 14

Telephone number: (1) 69.77.66

Title of the research contract:

Assessment of the radioactivity levels in Irish soils and their transfer into agricultural produce as a result of the Chernobyl accident.

List of projects:

1. Assessment of the radioactivity levels in Irish soils and their transfer into agricultural produce as a result of the Chernobyl accident.

Title of the project no.:

Assessment of the radioactivity levels in Irish soils and their transfer into agricultural produce as a result of the Chernobyl accident.

Head(s) of project:

Mr John D Cunningham

Scientific staff:

Dr Geraldine Mac Neill
Dr John O'Grady
Mr Jarlath Duffy

I. Objectives of the project:

The principal objective of the project is to study the levels of radioactivity in Irish soils and to estimate soil to plant concentration ratios for various types of agricultural produce. The concentration ratios will be computed primarily for Cs-137 although other radionuclides will be considered when present in sufficient quantities to allow an accurate assessment. The factors influencing transfer will be studied by considering the behaviour of deposited radionuclides in different soil types through a detailed examination of the soil characteristics.

II. Objectives for the reporting period:

The first objective was to complete measurements on the samples collected in 1987. A similar set of samples would then be collected during 1988. This would complete the sampling programme for the project. Measurements on the 1988 samples would commence immediately after collection.

III. Progress achieved:

1. Methodology

As described previously, eleven sites were chosen for sampling purposes. Sampling carried out at each of these sites in 1987 was repeated in 1988. This involved collecting samples of grass three times during the year (spring, summer and autumn), pasture and tillage soil twice (spring and autumn) and agricultural produce such as cereals and vegetables once (autumn). This concluded the 1988 sampling programme and in fact completed sampling for the two year project.

Soil samples collected during 1988 will be analysed for C.E.C., pH, carbon content, exchangeable potassium and total available potassium. Particle size analysis which was carried out on the 1987 samples will not be repeated. Gamma spectrometric analysis will be carried out on all soil, grass, cereal and vegetable samples. Grass samples from 1987 and 1988 will also be analysed for potassium content. This analysis may give some indication of the potassium growth of the grass. It is envisaged that all analyses will be completed by the end of March 1989.

2. Results

Concentration ratios (CRs) based on the 1987 samples have been computed for grass, cereals and potatoes. They are based on the ratio of the radionuclide activity per unit mass of plant to that of soil. The radionuclide concentrations of both plant and soil are expressed in terms of oven dried weights, except in the case of cereals for which the fresh or wet weight is used.

2.1 Grass

Concentration ratios for grass have been computed for the three sampling rounds in 1987. The calculated values represent the transfer of Chernobyl deposited caesium only. The measured soil activities were corrected by subtracting the contribution from weapons fallout. When this is done the CRs for Cs-137 and Cs-134 are essentially the same. This may indicate that the weapons caesium present in the soil is not transferred to the grass as readily as Chernobyl caesium.

The results for grass may be summarised by saying that CRs for grass during the months April to September 1987, at the eleven sites studied, lay in the range .05 to .47. Regression analysis has been used in an attempt to correlate CRs with soil parameter values. In most cases this has not provided any conclusive results. However there does appear

to be a significant correlation between concentration ratio and soil pH for the spring and autumn grass samples. This relationship does not hold for the samples taken during summer. It is proposed to do a potassium analysis on all of the 1987 and 1988 grass samples to try and establish stage of growth. This may provide an explanation for the seemingly anomalous results in the summer samples.

2.2 Potatoes

The concentration ratios for potatoes from the eleven sites ranged from 3×10^{-2} to 10×10^{-2}. Examining the soil chemical analyses, there is no obviously dominant parameter in determining transfer. It should be mentioned however that the low activities in all of the potato samples meant that there were large uncertainties in the measured values. This could have the effect of masking any trends that were present.

2.3 Cereals

2.3.1 Barley

Because most of the barley samples contained Cs-137 activities below the limits of detection (.2 Bq/kg), the concentration ratios have only been computed for three of the eleven sites. The range of values observed was from $<0.8 \times 10^{-2}$ to 2.9×10^{-2}. Based on so few results no correlation could be made between the transfer and the soil parameters.

2.3.2 Wheat and Oats

The concentration ratios for wheat ranged from 0.5×10^{-2} to 2.4×10^{-2}. The highest value was seen at site 1. This site had the lowest observed exchangeable potassium along with site 7. The CR at site 7 however, was at the lower end of the observed range, suggesting that other factors are important in the transfer process. Because wheat was not available at site 5 a sample of oats was collected instead. The CR was calculated to have been 3.3×10^{-2}.

3. Discussion

There was very little migration of Cs-137 down the soil profile during the first year after Chernobyl. Eighteen months after deposition more than 80% of the total caesium was contained in the first 5cm of soil.

Caesium deposited on soil during the weapons testing of the 1950's and 60's is still present in Irish soils. Rates of migration vary between soil types and locations.

Levels of Cs-137 in grass were seen to vary over a period of 6 months. Concentration ratios during this time ranged from .05 to .47.

Levels of Cs-137 in grain samples were generally less than 0.3 Bq/kg. This corresponded to concentration ratios ranging from $<0.8 \times 10^{-2}$ to 3.3×10^{-2}.

Levels of Cs-137 in potato samples ranged from 0.6 to 1.8 Bq/kg. The corresponding concentration ratios lay in the range from .03 to .13.

IV. Objectives for the next reporting period:

4. (i) Complete laboratory analysis on all samples collected during the second year of sampling which was completed in the autumn of 1988.

(ii) Integration and correlation of the 1988 measurements with previous results to provide an assessment of soil to plant concentration ratios for agricultural produce, mainly wheat, barley, potatoes and grass for different soil types.

(iii) Publication of a Board report detailing the results of the studies. The project terminates during 1989.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

An Teagasc
Johnstown Castle Research Centre
Wexford
Ireland

Department of Pure and Applied Physics
Trinity College
Dublin 2
Ireland

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Comitato Nazionale per la Ricerca e
lo Sviluppo dell'Energia Nucleare e
delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Contract no.: BI6-B-034-I

Head(s) of research team(s) [name(s) and address(es)]:

Dr. V. Damiani
Centro St.Amb.Mar., ENEA
Santa Teresa
Casella Postale 316
I-19100 La Spezia (SP)

Telephone number: 187/53.61.11

Title of the research contract:

Laboratory and field research on long-lived radionuclides in the marine environment.

List of projects:

1. Behaviour of Tc and Se in the marine environment.
2. Mechanisms involved in accumulation of transuranium in some compartments of the marine environment.
3. Descriptive models for circulation of radionuclides and transfer of radionuclides in the marine food chain.

Title of the project no.: 1

BEHAVIOUR OF Tc AND Se IN THE MARINE ENVIRONMENT

Head(s) of project:

Scientific staff: E.H. SCHULTE, R. DELFANTI, C. PAPUCCI

I. Objectives of the project:

A better knowledge on the environmental behaviour of long-lived radionuclides is necessary for the implementation of recent recommendations of ICRP, in particular for the assessment of collective dose commitments deriving from operations, such as programmed or accidental releases from nuclear plants and radioactive waste disposal, where transuranium nuclides and other long-lived radionuclides (Tc-99, I-129, Se-79, etc.) may represent an important contribution to the radiological inpact on man.

II. Objectives for the reporting period:

- Macro- and micro-distribution of Tc (Se) in benthic organisms;
- role of zooplankton in vertical transport processes of radionuclides in the water column;
- analysis of Tc-99 in biological matrices from the Mediterranean.

III. Progress achieved:

Macro- and Micro-distribution (autoradiography) of Technetium in benthic fish

Further results on the macro- and micro-distribution of Tc in Gobius sp. have been achieved using the long-lived isotope Tc-99 and autoradiographic methods. Fish accumulated Tc either from food (polychaetes) after Tc-uptake from water (~110Bq/l) or from polychaetes receiving an aliquot of Tc-99 injected into the body cavity (10µl, containing 370 kBq). The fish were fed daily with radioactive worms (Tc-uptake form water) for more than two months while fish receiving freshly contaminated worms (Tc-injection) were sacrified after three days when digestion was completed. All specimens were embeded in methyl-cellulose at -70 C and then sectioned (50-100 µm) with a cryomicrotome at -20 °C. The silices were exposed to KODAK X-OMAT AR Films (XAR-5) for some months.

The results obtained showed no differences in accumulation sites in the fish body between the two accumulation modes of Tc-99 by the organisms. These results confirmed also previous observations obtained in the same fish species using the γ-emitter Tc-95m. Althought Tc-95m-images were sufficiently clear, exposures to Tc-99 resulted in better images of Tc-distributions in the fish.

After the relative short exposure time to Tc-99, considerable amounts of this isotope were incorporated into bony structures (fin rays, scales i.e. whole body surface, cartilage of the head and vertebrae). The far most amounts of Tc-99 were still in the small intestine while the large intestine was little less contaminated. Liver, kindneys, and gills occupied the next grade showing similar quantities of Tc-99 accumulated. In sagittal cuts along the vertebral column the nervous system (brain, medulla) showed clearly higher technetium levels than the liver. The same holds for the retina of the eyes and the lenses. As in previous experiences no technetium traces could be detected, also after very long exposure times (9 months), in muscle tissues; thus the most important edible part of the fish remained uncontaminated.

Role of zooplankton in vertical transport processes of radionuclides in the water column.

As reported in the last year, following the Chernobyl accident, measurements of radioactive fallout in selected marine samples revealed peak concentrations of Chernobyl radionuclides in Ligurian Sea surface waters on day 4th and 5th of May 1986. After three weeks of contact with seawater only 2% of Cs-137 and 5% of Ru-103 present in the water column were in the particulate form and could be trapped on 0.45 um filters i.e. the radionuclide was adsorbed to particulate matter and then subjected to sinking processes finnaly reaching the sediment surface.

In this context we intended to study the role of zooplankton populations in enhancing the vertical transport processes of radionuclides via fecal pellets and their carcasses. Therefore, zooplankton has been sampled throughout the year bi-monthly in front of the La Spezia Gulf by means of a high-speed plankton sampler (7 knots), using plankton nets of 180 µm mesh. Samples were fixed in formalin for further γ-spectometry and biological sorting.

Gamma spectrometry measurements of zooplankton samples from July 1986 indicated clearly the presence of considerable amounts (59.5; 30.0; 90.2 Bq/Kg) of the respective radioisotopes Cs-137, Cs-134, and Ru-104, the latter ones originating from the Chernobyl accident. Further zooplankton samples collected during the reporting period are still to be processed and will be reported elsewhere.

Analysis of Tc-99 in biological matrices from the Mediterranean

The long-lived radionuclide Tc-99 may enter the geo-biosphere via different routes. Sources of Tc-99 to the environment are natural decay processes, fallout from nuclear weapon testing, uranium enrichment facilities, and discharge of effluents from nuclear fuel repocessing plants (accidental and/or programmed). Decay of the short-lived isomer Tc-99m, utilized for labelling radiopharamaceuticals, contributes only to a negligible extent to the amounts of Tc-99 present in the environment. However, the most important potential source of technetium is presented by radioactive waste disposal activities. In the Mediterranean technetium originates mainly from fallout, thus environmental concentrations of Tc-99 in different biological matrices may be found quite low.

Nevertheless, efforts were made to determine low Tc-concentrations in environmental samples using very highly efficient low-level beta-multicounter system consisting partly of a gas flow counter unit which incorporates five individual Geiger-Muller sample counter elements and a guard counter reducing drastically background radiation and resulting in a counting efficiency for Tc-99 of 42%.

During the reporting period various methods of the literature have been studied and tested for application to radiochemical preparation and analysis of different environmental and biological matrices. In the meantime samples have been collected from various sites along the Ligurian coast which are ready for radiochemical analysis of their Tc-99 content.

IV. Objectives for the next reporting period:

- analysis of Tc-99 in environmental matrices from the Mediterranean;
- remobilization and transfer of technetium and/or selenium from sediments to fish.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. S.W. Flower; International Laboratory of Marine Radioactivity, IAEA, Monaco MC.

VI. Publications:

E.H. SCHULTE, P. SCOPPA, A. SECONDINI - Biokinetics of Selenium in the benthic shrimp Palaemon elegans; C.I.E.S.M., Athens 17-22/10/1988

E.H. SCHULTE - Remobilization of technetium form sediments by polychaetes at the sediment-water interface, C.I.E.S.M, Athens 17-22/10/1988

R. DELFANTI, C. PAPUCCI - Characteristics of Chernobyl fallout in the Italian coastal marine environment. Presented at the "Internation Conference on Environmental Radioactivity in the Mediterranean Area", Barcelona, May 10-13, 1988.

Title of the project no.: 2

MECHANISMS INVOLVED IN ACCUMULATION OF TRANSURANIUM NUCLIDES IN SOME COMPARTMENTS OF THE MARINE ENVIRONMENT.

Head(s) of project: R. DELFANTI

Scientific staff: R. BONIFORTI, R. DELFANTI, I. NICCOLAI, C. PAPUCCI, C. PERONI, E. SCHULTE, A. ZATTERA.

I. Objectives of the project:

A better knowledge on the environmental behaviour of transuranium nuclides is necessary for the implementation of recent recommendations of ICRP, in particular for the assessment of collective dose commitments deriving from operations, such as programmed or accidental releases from nuclear plants and radioactive wastes disposal, where long-lived radionuclides may represent an important contribution to the radiological inpact on man.

II. Objectives for the reporting period:

- Field studies on soluble and particulate radionuclide fractions for evaluation of the processes taking place at the river-sea interface.
- Plutonium vertical profiles and inventories in sediments from Mediterranean continental shelf and slope (Ionian Sea).
- Mixing and sedimentation processes in deep-sea sediments.

III. Progress achieved:

METHODOLOGY

In summer 1987 a sampling campaign was carried out in the Taranto Gulf (Ionia Sea) for a study on the behaviour of transuranics and particle-associated pollutants in a canyon (Taranto Valley). The scope was to collect sediment cores from the continental shelf and slope and from the bottom of the canyon (-2000 m). The samples were collected in the western part of the Gulf, where a considerable amount of terrigenous material is transported by rivers.

Deep sea sediments were collected in 1984 and 1985 during the METEOR 69 and ESOPE (Etude des sediments oceaniques par penetration) cruises in the following Atlantic areas:
- Nordostatlantisches Monitoring Programm (NOAMP) Area
- Present NEA Dumpsite
- Great Meteor East (GME)
- Southern Nares Abyssal Plain (SNAP)

The sampling was carried out by a modified Reineck corer in the Taranto Gulf and in the first two atlantic areas and by a SIPAN corer with subsequent subsampling with a core barrel in the last two atlantic areas.
The cores were sectioned directly onboard into slices 1 cm thick. Selected sections of each core were analyzed for 239,240-Pu, 137-Cs and, for deep sea sediments, for 14-C.
239,240-Pu were separated from the matrix by a double anion exchange radiochemical procedure followed by electroplating and alfa spectrometry.
137-Cs was detected by non-destructive gamma spectrometry.
14-C measurements were carried out using a benzene liquid scintillation counting method.

RESULTS AND DISCUSSION

a) Shallow water environment

Two cores were analyzed for 239,240-Pu and 137-Cs; both of them were collected along the slope of the Taranto Valley at water depths of 450 m (core C6) and 800 m (core C5).

Both radionuclides are still measurable down to 27 cm for core C6 and to 24 cm for core C5. Maximum 137-Cs activities (14 Bq/kg d.w.) were found in the first centimeter of both cores. The highest 239,240-Pu activities (0.9 dpm/kg d.w. and 0.6 dpm/kg d.w. in cores C5 and C6 respectively) were determined in the 3 to 5 cm depth interval.
The vertical distributions of Plutonium and Cesium show similar patterns below the first 2-3 cm of sediment. At the sediment-water interface, the ratio 239,240-Pu/137-Cs is generally lower than in the deeper sediment layers, probably due to the deposition of Chernobyl 137-Cs.
Chernobyl contribution is particularly evident in the core collected at 450 m water depth, where the ratio

239,240-Pu/137-Cs is 0.02 in the first 2 cm and 0.06 along the core.

In both cases the radionuclide vertical profiles can be accounted for by mixing, or by sedimentation, or by a variable combination of the two. For each profile, only a maximum rate for a single process can be set when the rate of the other process is considered negligible. From fallout radionuclides penetration into the sediment column, a maximum sedimentation rate of about 0.5 cm/y can be estimated, even if the vertical profiles seem to indicate a prevailing influence of mixing phenomena.
Analyses of 210-Pb, presently being carried out, will give useful information to constrain one or the other variable.

b) Deep sea sediments.

For the characterization of the North-Atalantic areas, the vertical profiles of 14-C were analyzed. The use of a box-model allowed the calculation of the sedimentation rates (SR), the age of the particles arriving at the sediment surface (Tml) and the age of the mixed-layer (To).

Station	Water depth (m)	T ml (y)	T 0 (y)	SR (cm.ky-1)
NOAMP/Hill	4060	4665	1343	1.61
NOAMP/Talus	4520	2593	515	3.69
NEA Dumpsite/Plain	4710	2835	488	2.53
GME/Hill	5265	5422	509	1.08

The 14-C vertical distributions in most of the cores indicate a continuous uniform sedimentation regime during the last 10,000 years. The sedimentation rates were strongly influenced by the geometry of the sea-bottom and by the contribution of the eroded material from elsewhere.

Some evidence of horizontal influx has been recorded, as shown by the differences between the ages of the particles arriving at NOAMP/Hill and Talus, probably due to erosion processes.

As the GME sampling point was below the lysocline, the lower value of the sedimentation rate and age of the mixed-layer are probably related to dissolution of the foraminifera within the sediment and subsequent reduction of sediment volume.

IV. Objectives for the next reporting period:

- Plutonium and excess 210-Pb vertical profiles and inventories in sediments from a Mediterranean canyon (Taranto Valley - Ionian Sea).
- Mixing and sedimentation processes in deep-sea sediments.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. L. Scarpina AIRP (Italian Association for Radiation Protection). Bologna, Italy.

VI. Publications:

PAPUCCI, C. & DELFANTI, R. (1988) - 14-C and 239,240-Pu as tracers of sedimentation and mixing processes in North-East Atlantic sediments. In: Interim Oceanographic Description of the North-East Atlantic site for the disposal of low-level radioactive waste. OECD/NEA, Paris, in press.
DELFANTI, R., FIORE, V., LAVARELLO, O. & PAPUCCI, C. (1988) - Environmental Radioactivity along the Italian Coasts. In: Proceedings of the "International Conference on environmental radioactivity in the Mediterranean area. Barcelona, May 10-13, 1988. In press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor.

Univ. Catholique de Louvain
Halles Universitaires
Place de l'Université 1
B-1348 Louvain-la-Neuve

Contract no.: BI6-B-042-B

Head(s) of research team(s) [name(s) and address(es)]:

Prof J. Decallonne
Unité de Microbiologie
U.C.L.
Place Croix du Sud 2
B-1348 Louvain-la-Neuve

Telephone number. 010/47.36.72

Title of the research contract:

Description of the interactions and processes that are involved in Tc-99 movement and cycling.

List of projects:

1. Modelling of Technetium movement in soil
2. Study of the biogeochemical cycle of Tc-99 ; uncertainties associated with predictions

Title of the project no.: 1

Modeling of Technetium movement in soil.

Head(s) of project:

Prof. J. Decallonne.

Scientific staff:

Prof. J. Decallonne and Dr. C.N. Chiang.

I. Objectives of the project:

The first part of the projet was to investigate the possibility of quantitatively predicting the movement of pertechnetate in soils and stream.
The second part of the project was to investigate the possibility of quantitatively predicting the effect of Tc-99 as pertechnetate on microorganisms.
Later work will consist to fit together the two submodels, in order to describe quantitatively the movement of pertechnetate through soils and streams, as influenced by microorganisms.

II. Objectives for the reporting period:

Within the reporting period, the objectives of the work undertaken are as follows :

1- To further investigate, for identification, bacterial strains isolated from contamined lysimeter soil by the development of more accurate and less time consuming methods.

2- To study the Tc-99 (TcO_4^-) sorption to the soil, using small soil columns and under different soil conditions such as moisture, organic matter content, pH etc...

III. Progress achieved:

1. Methodology :

1-1. Bacterial strains identification :

Twenty four bacterial strains, isolated from the plough layer of a contaminated lysimeter soil - See project n°2 - were further investigated. First classical experimental observations were used to check their purity, their morphology, their motility and staining. Then an effort, to select the spores forming strains, was done. The growth of selected strains was tested on three media namely, Plate Count Agar, Tryptic Soy Broth and Lactobacillus Broth according to De Man, Rogosa and Sharpe (MRS), along with two temperatures, 25 and 37°C.

To achieve a rapid and accurate identification procedure for Bacillus isolates, a matrix of results, from tests in the API 20E and API 50CHB strips, was used. (Logan and Berkeley, 1984), totalizing 60 chemical tests. These observations were supplemented with gas chromatography analysis of bacterial cell-hydrolysates for their specific fatty acids composition. (Kaneda, 1977).

1-2. Tc-99 sorption to the soil :

To evaluate the Tc-99 sorption to the soil, representative samples of two soil types were mixed with sand in small columns of 20 cm high and 2 cm in diameter. Main characteristics of the soils are listed in table I- The appropriate amounts of sand to be added to each soil, to achieve the desired moisture conditions, were calculated following Lueking and Schepers (1986), adjusting original sand and organic matter contents of each sample. The columns were first leached with a saline solution containing, per liter, 2g of KH_2PO_4; 0.5g of NH_4Cl; 0.03g of $MgSO_4$; 0.005g of $FeSO_4$ and 0.01g of $CaCl_2$; the columns were tightly covered with rubber stopper and incubated at 25°C for 2 weeks (Henrot, 1988). Then, a solution of $NH_4^{99}TcO_4$, 20ml with 925 Bq/ml, is added to the columns, prior to their re-incubation. Excess of the solutions, saline and Tc-99, are vacuum extracted with a suction of 80 kPa. until the desired moisture conditions are reached. For the two soils under experiment, they were : 90 % of water filled porosity (WFP) for the so called aerobic condition and 120 % of WFP for the anaerobic condition. Control of water contents was carried out by weighing the columns before and after each leaching and during the incubation. After chosen period of time, 0, 1, 2, ...7 days, a set of columns, representing each soil at the two moisture conditions were leached with 50 ml of $CaCl_2$, in increments of 10 ml. Tc-99 activity was measured in the leachates by liquid scintillation counting (Beckman LS78000).

2 Results :

2 1. Bacterial strains identification :

Ten strains, with confirmed sporulation ability, were under intensive cross checking for accurate identification. Four out of them can be assumed as correctely identified. They are mostly long rod shaped and Gram positive Bacillus, namely Bacillus *thuringensis*, B. *cereus*, B. *anthracis* and B. *mycoides*. Figure 1 presents typical fatty acids distribution, obtained by gas chromatography analysis for B. *cereus* cell hydrolysate.

2-2. Sorption of Tc-99 to the soils :

Figure 2 shows the time course of the Tc-99 sorption to the soils. For the first soil, Habay-la-Vielle, sorption in aerobic condition is characterized by a rate constant of 0.1 d^{-1}, corresponding to a half-reaction time of 7 days, whereas the anaerobic condition is characterized by a rate constant of 0.6 d^{-1} with a half-reaction time of 1 day, eventhough the maximum amount of sorbed Tc-99 are less important, 120 Bq/g of dry soil instead of 202 Bq/g. An opposite figure is found for the Beauvechain soil which shows a higher rate constant, 0.37 d^{-1} with 2 days as half reaction time, for aerobic condition and smaller sorbed amounts (68 Bq/g) when compared to the anaerobic condition, 0.14 d^{-1} and 5 days as half-reaction time. Once the soils are compared to each other, the first observation is the more important amounts of sorbed Tc-99 found in the first soil. As it can be seen in Table I, the soils used in this study differ mainly by the organic matter content, consequence of the respective pH values. This initial organic matter content can be an explanation of the differences found in the two soils in terms of concentrations of Tc-99 sorbeb to the soils. This result is consistent to the data reported by Henrot (1988), assuming that Tc-99 sorption can be equated to the Tc complexation with the soil organic matter. This effect is theoretically enhanced when the Tc-99 is maintained at a lower oxidation state. In our experiment, the latter condition is met, when the water filled porosity is higher than 100 %. Thus, the sorbed Tc-99 concentrations measured in the Beauvechain soil (204 Bq/g) are close to those observed for the first soil, when anaerobic conditions prevailed.

3- Discussion :

The procedure developped, for bacterial strains identification, in this reporting period, has shown better possibilities for the knowledge of micro-organisms able to accumulate/or reduced the Tc-99. The gas chomatography analysis for bacterial fatty acids composition represents a performing tool, at least in terms of less time-consuming technique, to reduce the uncertainty in the identification procedure.

The small columns used in the Tc-99 sorption to the soil study , represent a method easy to handle to provide data. By adding appropriate amounts of sand, desired moisture conditions or oxidation state can be achieved, and the expression of these conditions in terms of water filled porosity, seems to be pratical enough to express the oxygen diffusion. The remaining question is how realistic these experimental conditions are for the field.

Table I : Main characteristics of the studied soils :

Soils :	Habay-la-Vieille	Beauvechain
Location :	Ardennes	Brabant
pH (H_2O):	5.1	6.9
Organic Matter (%) :	4.5	1.9
Sand, Loam and Clay (%) :	19.5; 62; 18.4	11.1; 72.3; 16.6
C.E.C (Meq/100g)	15	10.5

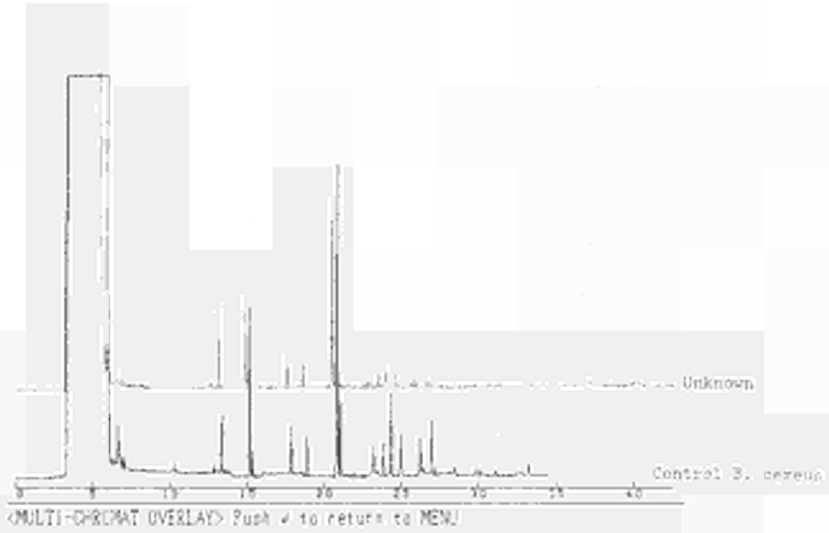


Figure 1 - Chromatogram of methylated fatty acids obtained from bacterial cell-hydrolysate Identification: Bacillus cereus

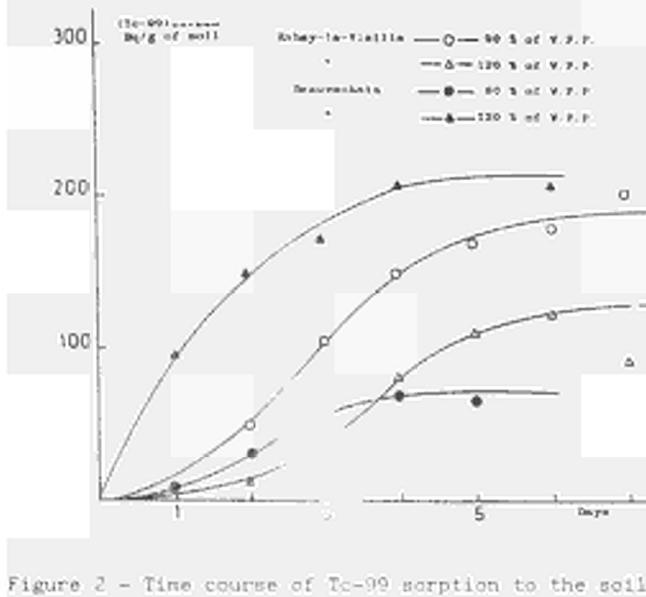


Figure 2 - Time course of Tc-99 sorption to the soils

IV. Objectives for the next reporting period:

Since the part achieved in the indentification of baterial strains concluded rather satisfactorily, for the next reporting period, the planned work should be as follows :

1- Using the matrix of results tested this period and the gas chomatography, systematic work will be carried out, to adequately identify, at least, the existing strains (24), with a special attention for the anaerobic strains.

2- Tests of their ability to accumulate and/or reduce the tc-99 will be performed as soon as a strain is identified correctely.

In terms of Tc-99 sorption study, the small columns will be used to experiment different soils conditions and to supplement the precedent point.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

-Prof. Myttenaere, Catholic University of Louvain, Belgium.
-Oak Ridge National Laboratory- ORNL: Dr. Auerbach, Hoffman, Blaybock USA
-Prof. Pieri J. : University of Nantes. France.
-Prof. Cremers A., Katoliek Universiteit Leuven. Belgium

VI. Publications:

Title of the project no.:

Study of the biochemical cycle of Tc-99 - Uncertainties associated with prediction.

Head(s) of project:

Prof. C. MYTTENAERE

Scientific staff:

Cogneau, M., Dehut, J.P., Deprinc, D., Hauzeur, J., Sombre, L., Van Laer,S. Vandecasteele, C.M.*

I. Objectives of the project:

The main objective of the project is to reduce the uncertainty which affects the Tc behaviour in the terrestrial environment.
In 1988 the scope of the project was enlarged and the methods were applied to non grass type plants (coniferous ecosystems).
The study was also extended to another isotope (Cs-137) which has raised the main important questions after the Tchernobyl accident.

II. Objectives for the reporting period:

The following studies were carried on in 1988.

Technetium-99

- Study of the long term availability of technetium deposited on soil in the open lysimeters (validation of the ageing phenomena model).
- Assessment of the interception and retention of Tc deposited on spruces.
- Study of the degradation of the bio-incorporated Tc.

Caesium-137

- Study of the absorption and retention of Cs-137 by spruces.

* Scientific collaborator

III. Progress achieved:

1. Validation of the long term behaviour model in soils.
The LLN model was validated by data collected in 1988.
Figure 1 gives the evolution of the transfer factors since the beginning of the observations.
The values of the parameters have been confirmed by the last observations.
In 1988 the transfer factor from soil to grass (cpmg DW/cpmg D soil) was as expected very low (± 5); 26% of the initial activity remained in the soil 6 years after contamination.

2. Availability of the bioincorporated Tc.
The transfer factors obtained in minilysimeters filled with soils contaminated with leaves of poplar and needles of harches are given by figure 2.
The transfer factor are now of the same value than those observed in the open lysimeters. The highest availability was observed for the poplar material; in these conditions 29% of the radioactivity was exported by grass against 11% for the needles of harches (Figure 3).
In these conditions (as in the open lysimeters) soil must also be considered as a long term sink for Tc-99 radioactivity.

3. Interception of TCO_4 by spruces.
Water contaminated by a mixture of Tc-99 (0,5 uCi/l) and Tc-95m (5 uCi/l) on the pertechnetate form was sprayed on a spruce of 8 years old and samples were collected (needles, shoot) before and after a non contaminated rain.
Contrary to what happens after a root contamination, the contamination of the needles was clearly related to their situation in the tree and not their age; a rain may export more than 50% of the radioactivity deposited.
The study of the distribution of the radioactivity in the needles using different solvents water, water + Tc chloroform, showed that the waxy coating of the needle plays a very important role in the retention of the deposited Tc.

4. Transfer of Tc from soil to trees.
Poplar and harches are cultivated in minilysimeters. The superficial layer was contaminated in Tc-99 and plant material regularly collected.
4 years after the contamination the water soluble fraction of the soil contains respectively 8,9 and 3.4% of the total content of the soil for the harches and poplar lysimeters. The transfer factors obtained for the leaves of the two species are 1.9 (harches) and 2.1 (Poplar).

5. Roles of direct and indirect contaminations in the Cs-137 transfer in a coniferous ecosystem. *

Two 8 years spruces were transplanted in the open lysimeters. Before transplantation Cs-134 thermo-generated aerosols were deposited on the trees (aerosols generated at about 2000°C by a laboratory simulator) and the surface layer of the soil was contaminated in Cs-137 (1 mCi m-2) Needles are regularly sampled as well as soil and percolates. The experiment is in course and the results given in the next report.

* The work is partly supported by the Belgian Ministery of Health.

FIGURE 1 : FACTEURS DE TRANSFERT

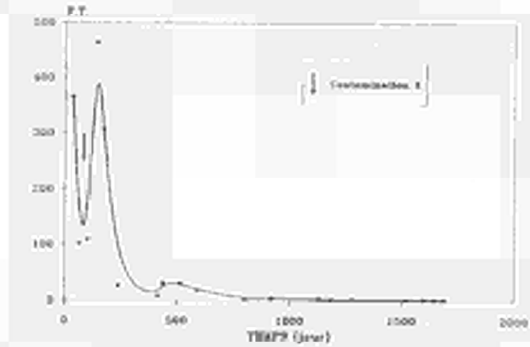


FIGURE 2 : *EXPERIENCE MELEZE-PEUPLIER*
FACTEURS DE TRANSFERT

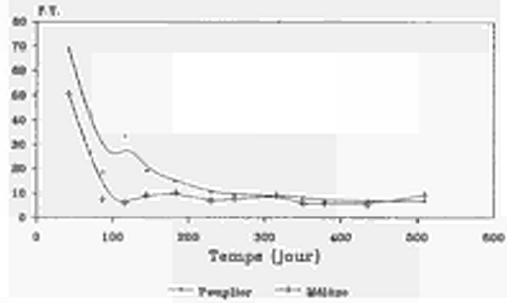
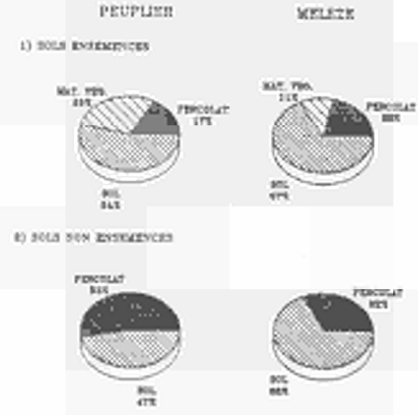


Figure 3.

BILAN DES EXPORTATIONS



IV. Objectives for the next reporting period:

- Validation of the long term model for Tc-99.
- Study of the long term transfer of bioincorporated Tc-99.
- Study of the direct contamination of non grass type plants by Tc (spruces).
- Study of the biogeochemical cycle of Cs-137 in forest ecosystems.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Prof. CREMERS, KUL, Leuven, Belgium.
- Pacific Nortwest Lab. Richland, Washington, Prof. WILDUNG, Dr. CATALDO.
- Prof. SCHELL, University of Pittsburgh, Pensylvania, USA.
- Dr. HENROT, Villanova University, Pensylvania, USA.
- Prof. GARREC. INRA, Champenoux, Nancy, France.

VI. Publications:

SOMBRE, L., CARRARO, S., MYTTENAERE, C. Transfert du Cs-137 dans une chaîne alimentaire d'eau douce simplifiée eau-algue verte (Scenedesmus-obliquus) Mollusque filtreur (Dreissena polymorpha). Ann. Soc. Belge Radioprotection, 12, 2-3 p. 205-28 (1987).

SOMBRE, L., CARRARO, S., MYTTENAERE, C., Contamination d'une algue verte d'eau douce (Scenedesmus obliquus) par des radionucléides typiques des rejets d'une centrale PWR: Culture dans un turbidostat. Ann. Soc. Belge Radioprotection, 12, 2-3 p. 157-70 (1987).

RONNEAU, C., FAGNIART, E., FONSNY, K., ANDRE, P., Contamination des écosystèmes forestiers par le radiocésium. IV Symposium Int. Radioécologie, Cadarache, Impact des accidents d'origine nucléaire sur l'environnement, CEA, IPSN, DERS, 14-18 mars 88.

VAN LAER, S., FONSNY, K., MYTTENAERE, C., Etude du recyclage du Tc-99 incorporé dans la matière végétale forestière. IV Symposium Int. Radioécologie, Cadarache, Impact des accidents d'origine nucléaire sur l'environnement, CEA, IPSN, DERS, 14-18 mars 88.

DESMET, G., MYTTENAERE, C., Considerations of the role of natural ecosystems in the eventual contamination of man and his environment. Jl. Env. Radioactivity, 6, p. 197-202 (1988).

RAYYES, A.A., RONNEAU, C., APERS, D., MYTTENAERE, C., Ecological behaviour of thermo-generated ceasium aerosols. Doklady Academic Naouk, UK SSR,8 , p. 76-9 (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB- London SW1Y 4QP

Contract no.: BI6-B-046-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr R.G. Derwent
Env.& Med. Sciences Div.
AERE
Harwell, Didcot
GB- Oxon OX11 ORA

Telephone number: 0235/241.41

Title of the research contract:

Distribution and transfer of radionuclides in terrestrial and sea environments.

List of projects:

1. Comparison of radionuclide deposition to vegetation
2. Comparative study of soil to plant transfer of Neptunium, Plutonium, Americium and Curium
3. Exchange of radionuclides between the sea and atmosphere.

Title of the project no: 1

Comparison of radionuclide deposition to vegetation

Head(s) of project:

Dr R G Derwent

Scientific staff: P A Cawse
S J Baker
P Burton
B Sykes

I. Objectives of the project:

To examine the influence of geography and climate on the current concentrations of Sr-90, Am-241, Pu and gamma-emitters including Cs-134 and Cs-137 in atmospheric deposition, in soil and in vegetation, and to apply these 'baseline' data to parallel studies in the environment of nuclear installations. The continuous measurements will allow seasonal effects to be established and sea to land transfer of actinides at coastal locations to be assessed. An associated collaborative study is being made in France by CEA Cadarache extending southwards to 44^{o}N (see Section V) for comparison with the measurements in Gt. Britain which extend northwards to 58^{o}N.

II. Objectives for the reporting period:

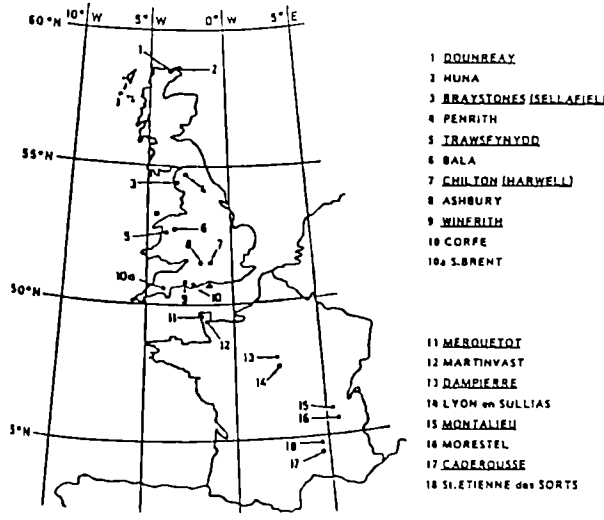
(a) to maintain continuous sampling at 11 field plots of atmospheric deposition, ryegrass and lucerne, including sampling of herbage grown in standard soil with low radionuclide content, to indicate the importance of atmospheric deposition of radionuclides and to follow the fate of Chernobyl fallout.
(b) to complete analysis of samples collected in 1988 and to commence analysis of 1989 samples for radionuclides; Na and Tl are analysed in rainwater to assess deposition of sea spray and soil dust.
(c) to collaborate with CEA Cadarache on co-ordination of the project with measurements in France.

III. Progress achieved:

1. Methodology

The routine collection of total (wet and dry) deposition, ryegrass and lucerne has proceeded at field plots in Gt. Britain for comparison of radionuclide concentrations with France (Fig.1). The experimental plots are located both near to and distant from nuclear installations. The vegetation was harvested at the end of May 1988 and again in September: ryegrass and lucerne are selected as test species to examine the differences in retention of airborne particulates containing radionuclides by narrow-leaved and broad-leaved foliage, in addition to examination of differences in root uptake. The root systems of these crops are in contrast, with ryegrass being fibrous-rooted whereas lucerne is tap-rooted, penetrates to greater soil depth and is drought resistant. The deposition collector at each plot is changed when vegetation is sampled to provide quantitative data on interception by foliage of the a ospheric input inventory of radionuclides. A standard fertiliser treatment is applied to the grass which receives N-P-K in March and N only in July. Lucerne receives P-K fertiliser in March and again in autumn. The soil pH is kept at pH 6.5 minimum by addition of calcium carbonate.

Soil sampling and analysis was made at the start of the project in 1985/86 and again after the Chernobyl accident in April 1986. Over the period autumn 1987/spring 1988, soil profiles were sampled from permanent grassland at each field plot location to assess the migration of Cs-134 and Cs-137 to 0.5 metres by analysis of soil from 12 depth intervals.



—— Underline denotes that plot is located at or very near to nuclear installation The remainder are distant.

FIG 1 LOCATION OF FIELD PLOTS FOR MEASUREMENT OF RADIONUCLIDES IN DEPOSITION AND VEGETATION

2. Results

(a) Deposition measurements

Deposition of radiocaesium continued to decrease, towards pre-Chernobyl levels. The ratios of winter/summer atmospheric deposition for Pu and Am-241 varied between plot sites according to site specific conditions and sources; at Braystones (near Sellafield) the deposition of Pu-239+240 and Am-241 over winter increased by 7 to 10 times and a corresponding increase of 4-fold in Na deposition over winter suggests that sea spray aerosol is mainly responsible for this transfer mechanism.

(b) Vegetation analysis

It is clear that soil to plant transfer of Cs-134 and Cs-137 by the deeper-rooted lucerne is much less than for ryegrass; at plots in N.Britain which received substantial Chernobyl fallout, analysis showed:

Field Plot	Bq/kg dry weight in herbage			
	Cs-134		Cs-137	
	Ryegrass	Lucerne	Ryegrass	Lucerne
Dounreay	1.5	0.24	8.0	1.7
Huna	0.35	0.24	2.9	0.72
Braystones	5.1	1.1	16.0	4.5
Penrith	1.7	<0.4	5.8	0.36

In contrast, Sr-90 concentrations in lucerne were generally greater than found in ryegrass. With regard to foliar interception, the data from Chilton plot showed that ryegrass retained 93% of the atmospheric input of Pu-239+240 over the growing season compared with 23% for lucerne.

(c) Soil profile measurements

At plots receiving substantial Chernobyl fallout (in N. Wales, Cumbria & Scotland) the concentrations of Cs-137 in surface layers (0-4 cm depth) of permanent grassland soils near to nuclear installations were in the range 96-390 Bq/kg compared with 36-210 Bq/kg at distant locations. In the south of England, which received relatively little Chernobyl fallout, the plots near nuclear sites showed Cs-137 concentrations of 17-32 Bq/kg (0-4 cm) whereas distant locations gave 5-16 Bq/kg, an exception being in S. Devon where high annual rainfall resulted in surface soil concentrations of 55 Bq/kg by accumulation of Cs-137 from nuclear weapons fallout. The ratios of Cs-137/134 increased with depth, owing to pre-Chernobyl deposition of Cs-137 at both nuclear and distant sites. Surface soil concentrations of Cs-137 were an order of magnitude greater than found below 8 to 10 cm depth, both at nuclear and distant locations; regardless of concentration differences, averages for each group showed that 60% of the accumulated deposition of Cs-137 from all sources is now retained in the top 8 cm of the profile.

3. Discussion

The results have contributed to a time series of data that reveal both seasonal differences and variation in concentrations of radiocaesium and actinides in environmental media at nuclear and distant sites. Complementary data is obtained by the French CEA. The Chernobyl fallout remains highly concentrated in surface soil layers and is available for uptake by shallow-rooted vegetation. At some plots near nuclear sites, interception and retention of Pu and Am-241 by foliage continues to be the main transfer pathway leading to elevated concentrations in herbage.

IV. Objectives for the next reporting period:

(a) to maintain the sampling programme for atmospheric deposition and vegetation until mid-1989 according to the established schedule, and to continue with the analysis of gamma-emitters and actinides. The fate of Cs-134 and Cs-137 derived from Chernobyl fallout will continue to be followed.
(b) to derive an inventory of radionuclide deposition to soil and vegetation, including seasonal (summer/winter) comparisons, and to examine the site specific differences, and the climatic influence.
(c) to collaborate with CEA Cadarache on progress of the measurements and interpretation of results.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]

Centre d'Etudes Nucleaires de Cadarache, Service d'Etudes et de Recherches sur l'Environnement: Chef, A. Grauby, BP no. 1, 13108, Saint-Paul-lez-Durance, France.

Collaboration is made with A. Grauby, J. Delmas and C. Colle. Discussion took place with CEA Cadarache in spring, summer and autumn 1988 during exchange visits.

VI. Publications

Cawse, P.A., and Colle, C. (1988) Comparison of radionuclide deposition to soil and vegetation. In, Proceedings of the 4th International Symposium on Radioecology, Volume 1, D-76 to D89, Cadarache, France, March 1988.

Title of project no: 2

Comparative study of soil to plant transfer of Neptunium, Plutonium, Americium and Curium

Head(s) of project:

Dr R G Derwent

Scientific staff: P A Cawse
S J Baker
P Burton
B Sykes

I. Objectives of the project:

The objectives are to provide improved data on soil to plant transfer of actinides following contamination of different soil types that occur in Gt. Britain and in France. The effect of ageing in soil of actinides will also be determined with respect to plant uptake and the distribution of actinides in soil compartments. The information gained will improve the accuracy of radiation dose estimates to man from the ingestion pathway.

II. Objectives for the reporting period:

(a) to analyse crop samples collected from the Np-237, Am-241 and Cm-244 tracer experiments in 1987 and to continue the harvesting programme in 1988. The influence of ageing on uptake of tracers is to be examined by the soil to plant transfer factors obtained during the course of the study over three years, using ryegrass and lettuce as test plants.
(b) to collaborate with CEA Cadarache, who are carrying out a series of experiments on uptake of Np-237 and Am-241 by additional crop plants using the same organic (fen) soil from Gt. Britain, and French soils (brown acidic and brown calcareous earth).

III. Progress achieved:

1. Methodology

In July 1986, the tracer experiment was started with Np-237 and Am-241 and in July 1987 with Cm-244 applied in nitrate form to containers each holding 15 kg dry weight of soil. Soil types used are an organic (fen) soil, brown earth (acidic), brown earth (neutral), and brown calcareous earth. Ryegrass is used as test plant to provide continuous samples from all soil types whereas lettuce is grown each year in the organic soil. The effect of soil sterilisation and organic fertiliser (sewage sludge) is also being examined with respect to tracer uptake by ryegrass. Analysis of samples collected in autumn 1987 and spring 1988 has proceeded and further crop samples have been obtained in summer 1988. The total removal of tracer by continued cropping is recorded.

2. Results

In the acid brown earth (pH 5.8) uptake of Np-237 and Am-241 by ryegrass was considerably elevated for three seasonal harvests, the soil to plant transfer factors being up to 3 orders of magnitude greater than for herbage grown in organic soil (pH 6.9). So far, although no general trends in transfer factors have appeared for all soils, the neutral brown earth (pH 7.0) has shown a steady decrease in uptake by ryegrass. Further, it is noted that the organic soil shows greater variability from season to season in transfer factors for Np-237 and Am-241 than the brown earths.

Concentrations of tracers in ryegrass and transfer factors obtained from the May 1988 harvest (i.e. winter 1987/88) are as follows:

	Np-237		Am-241		Cm-244	
Soil type	Bq/kg	T.factor	Bq/kg	T.factor	Bq/kg	T.factor
Organic (fen)	20.5	2.8×10^{-3}	2.1	1.1×10^{-4}	1.2	6.3×10^{-5}
Sterilised organic (fen)	11.1	1.5×10^{-3}	4.5	2.4×10^{-4}	0.40	2.1×10^{-5}
Brown earth (neutral)	118.0	1.6×10^{-2}	3.6	1.9×10^{-4}	5.2	2.8×10^{-4}
Brown earth (acidic)	2024	2.7×10^{-1}	111	6.0×10^{-3}	1.6	-
Brown calcareous	42	5.7×10^{-2}	1.9	1.0×10^{-4}	3.1	-

Transfer factors are calculated on a dry weight plant/soil basis. Tracer concentrations are 18.5 kBq/kg dry weight soil for Am-241 and Cm-244 and 7.4 kBq/kg for Np-237.

The sterilisation of the organic soil resulted in reduced uptake of Np-237 and Cm-244 and may indicate suppression of soil micro-organisms associated with the root rhizosphere zone; this effect is not apparent for Am-241.

Application of sewage sludge to acidic brown earth increased uptake of Am-241 by ryegrass up to 4 fold, and up to an order of magnitude when added to organic soil. For Cm-244, ryegrass in these soils shows a similar response, although less enhanced than Am-241. In the case of Np-237, although initial harvests (in 1987) from organic soil gave order of magnitude increases in uptake by ryegrass in response to sewage addition, this was not sustained.

The total removal of tracers in the course of ryegrass harvests from July 1986 to May 1988 is 322 Bq Np-237 and 14 Bq Am-241 for the acidic brown earth compared with only 4.6 Bq Np-237 and 0.44 Bq Am-241 from the organic soil.

3. Discussion

The soil to plant transfer of Np-237, Am-241 and Cm-244 is shown to be greatly influenced by soil type and amendment. In addition, sterilisation of soil can also affect tracer uptake. The observation that acidic brown earth enhances uptake of actinides (at pH 5.8) indicates that studies are needed using soils in the very acid category (pH 4 to 5) with fescue and/or other acid-tolerant species as test plants. Such acid soils are predominant in large areas of Gt. Britain and France. Transfer factors derived from the present studies will be contributed to the IUR data bank; in particular, information on Cm-244 uptake is needed.

IV. Objectives for the next reporting period:

Analysis of vegetation samples collected in 1988 will be completed for comparison with previous results. Further samples of ryegrass and lettuce will be grown in 1989, the lettuce being re-sown in Spring. Soil samples will be taken after the final harvest to assess the distribution of Np-237 and Am-241 in soil fractions. Studies on Cm-244 uptake will continue for 3 years until mid-1990 according to the established experimental programme and results will be discussed with CEA Cadarache.

V. Other research group(s) collaborating actively on this project [name(s) and addresses(es)]:

Centre d'Etudes Nucleaires de Cadarache, Service d'Etudes et de Recherches sur l'Environnement: Chef, A. Grauby, BP No.1, 13108, Saint-Paul-lez Durance, France.

Collaboration is made with A. Grauby, J. Delmas and C. Colle. Experimental data for uptake by other crops of Np-237 and Am-241 is now obtained by CEA Cadarache using the same soil types and is being compared with Harwell data which is complementary.

VI. Publications:

None.

(Experimental work is still in progress to obtain the required time series of data).

Title of the project No: 3

Exchange of radionuclides between the sea and atmosphere

Head(s) of project: J A Garland

Scientific staff: W A McKay
M I Walker
J Cloke

I. Objectives of the project:

To investigate the mechanisms by which artificial radionuclides in the sea can be converted into aerosol in the atmosphere above the sea surface, and to study this aerosol as it is transferred to land. The present project is concerned with laboratory and field studies in roughly equal proportions, the associated project from France deals mainly with laboratory studies.

II. Objectives for the reporting period:

Gain a greater understanding of:

(1) the variation in beach aerosol size distribution and concentration with wind speed and distance from the surf zone.

(2) the importance of seawater dissolved organic content, on aerosol flux and sediment loading on aerosol particulate content, and thus actinide sea to air transfer.

Objective 2 will be carried out in collaboration with the University of East Anglia.

III. Progress achieved:

(1) Field Programme

An assembly of instruments was used at Eskmeals on the Cumbrian coast (14 km SE of Sellafield) to characterise the seaspray aerosol during a number of sampling periods including a half tidal cycle. The results of optical measurements, using a laser phase doppler system developed at Harwell, showed a peak in the size spectrum at 15 μm, with an extended tail of much larger particles. A cascade impactor was used to obtain information on the composition of aerosol particles below 10 μm as a function of particle size, and rod impactor system was used for larger particles. Results are still being analysed, but clearly indicate:

(i) a strong dependence of sea spray concentration with wind speed,

(ii) a decrease of about two orders of magnitude in the concentration of large spray drops as the edge of the water recedes from 13 m to 200 m from the instruments.

(iii) a major fraction of aerosol mass associated with particles greater than 50 μm diameter.

(2) Laboratory Programme

(1) Monthly measurements of dissolved organic carbon (DOC) and surfactant activity have been carried out on seawater samples from the Irish Sea (Menai Straits) and North Sea (Gt. Yarmouth). Laboratory studies of the generation of spray by bubble bursting has been carried out using these seawater samples.

Although trends are apparent in both DOC and surfactant activity, there is no obvious relationship with aerosol rate. Experiments using artificial seawater and DOC concentrations <1 mg C l^{-1} showed enhanced aerosol flux with increasing surfactant activity due to the flux of fine bubbles reaching, and bursting at, the surface being increased.

It would appear that in near-shore coastal waters where DOC concentrations are typically >1 mg C l^{-1}, bubbles are saturated with respect to surface-active organics, thus an increase in DOC levels has little effect on aerosol generation.

An increase in the thickness of a bubble's organic coating could, however, influence the amount of particulate associated actinides a bubble transfers to the surface, by increasing its drag and thus scavenging time. A seasonal increase in DOC could thus increase the amount of actinides the aerosol carries without influencing aerosol flux.

(2) The importance of suspended particulate loading (in the seawater) on aerosol particulate content is being investigated using silica and alumina particles of known size in the laboratory bubbling system. A reproducible method has been developed and results should be generated shortly.

IV. Objectives for the next reporting period:

Gain a greater understanding of actinide sea to air transfer through (1) determining the droplet size and particulate content of the sea spray aerosol for a wide range of wind speeds (2) determining the influence of seawater particulate loading and dissolved organic content on particulate enrichment in the aerosol.

V. Other research group(s) collaborations actively on this project [name(s) and address(es)]:

Professor P S Liss
School of Environmental Sciences
University of East Anglia
Norwich
NR4 7TJ
England

Dr Y Belot
Commissariat a l'Energie Atomique
Department d'Etudes et de
Recherches en Securite
Boite Postale 6
92260 Fontenay-aux-Roses
France

VI. Publications:

McKay, W.A., Pattenden, N.J. and Cambray, R.S. (1988) Some of the processes involved in the transfer of plutonium and americium from sea to land. Seminar on "The cycling of long-lived radionuclides in the biosphere: observations and models". 15-19 September 1986, Vol.2. CEC.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-B-199-NL

Netherlands Institute
for Sea Research
P.O. Box 59
NL-1790 AB Den Burg

Head(s) of research team(s) [name(s) and address(es)]:

Dr. E.K. Duursma
Netherlands Institute
for Sea Research
P.O. Box 59
NL-1790 AB Den Burg

Telephone number: 02226-541

Title of the research contract:

Biological and geochemical investigation in relation to the deep sea dumping of low level radioactive waste.

List of projects:

1. Biological and geochemical investigation in relation to the deep sea dumping of low level radioactive waste.

Title of the project no.: 1
Biological investigation of the NEA-dumpsite in relation to the deep sea dumping of low level radioactive waste.

Head(s) of project:
Dr. H.J. Lindeboom
Dr. E.K. Duursma
Drs. M.S.S. Lavaleye

Scientific staff:
Drs. M.S.S. Lavaleye

I. Objectives of the project:

1. To identify possible biological processes that influence the transport of radionuclides after their eventual release from the waste canisters at the NEA-dumpsite. To this purpose a thorough knowledge of the benthic fauna and the deep-sea foodweb is necessary. These topics will be studied at the dumpsite and also along a transect northward of the site.
2. To see if there is a transport of radionuclides from the waste canisters to organisms. For this purpose analysis of radionuclides in the megafauna collected at the site will be carried out.

II. Objectives for the reporting period: 1 Jan. - 31 Dec. 1988.

- Participate in two expeditions to the NEA-dumpsite and surroundings, to collect bottomfauna for biological research and radionuclide analyses.
- Starting the biological research and the radionuclide analyses of megafauna.

III. Progress achieved:

Methodology:

During two expeditions in March-April and June-July 1988 to the NEA-dumpsite and a downstream transect north of the dumpsite, benthic samples of meio-, macro- and megafauna were collected. For meiofauna sampling a Scripps-boxcorer as well as a Multiple corer were used to test the hypothesis that the last apparatus is superior in collecting meiobenthic fauna to the Scripps-boxcorer. Undisturbed sediment samples for macrofauna research were sliced horizontal to study the vertical distribution of the infauna, and sieved over 1 mm and 0,5 mm screensizes. To study the small scale distribution 10 boxcore samples were taken at one spot.

Megafauna had to be collected during the second expedition with a 3,5 m Agassiz trawl with photocamera. Because of bad weather and technical problems only one succesful trawl catch was hauled in nearby our most northern station.

Results and Discussion:

In the second half of 1988 the samples were available and the research was started. Of the 4 topics of this project the interim results are discussed here:

Biology:

1. Comparison of the meio- and macrobenthic community of the NEA-dumpsite with other nearby deep-sea areas, especially those situated down-stream. The first results show that the 4 stations on the transect have a more or less equal Metazoan macrofauna density. The differences are even smaller than those between the stations of the DORA-project (1982-1986). Polychaetes dominate in density the other animal groups. Striking is that in the two most northern stations Echinoidea were present, animals which were never found in the boxcore samples of the dumpsite. This indicates a change in composition of the macrofauna community. Preliminary results of the comparison between Scripps-corer and Multiple-corer suggest strongly that the Multiple-corer collects the deep-sea meiofauna much better. Not only agile animals like nauplii and Nematoda, but also unexpectedly the Foraminifera are much more abundant. Comparison with data of the DORA-project shows that densities of Nematoda, nauplii and Copepoda fall within the same range. However, densities of Foraminifera are now much higher, which can partly be explained by the fact that the DORA-project was mainly focused on Metazoan-fauna.

2. Study of the deep-sea foodweb.
   Literature and taxonomic research on the animals collected in and near the dumpsite confirms that the greater part of the animals are predominantly deposit feeders. The work on a total review, specialised on the dumpsite, of the different deep-sea benthos groups and their trophic levels was started. Cooperation with the Natural History Museum at Leiden is already assured for the Cnidaria.

3. Quantitative measurements of megafauna.
   This research could not be started because of a lack of samples. There is a chance this could still be done in 1989.

Radionuclide analysis:

4. Because of the bad weather and technical problems no megafauna at the planned 4 stations was caught during the 1988 expedition. Only one good catch at a shallower depths, NE of our northern station was hauled in, which proved that the trawl worked well. In 1989 we will again try to get megabenthos, so that radionuclide analysis can be carried out, to verify if there is an actual transport of radionuclides from the waste canisters to the benthic organisms.

IV. Objectives for the next reporting period:

- Collection of megabenthos for radionuclide analysis at 4 stations at a transect from the dumpsite to the north.
- Completion of the biological research on the distribution of the benthos, comparison of the deep-sea fauna and the study of the foodweb.
- Publish the results in a final report at the end of the year.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Coordinated Research and Surveillance Programme (CRESP), coordinated by the Nuclear Energy Agency (NEA).
- Institut für Hydrobiologie und Fischereiwissenschaft (IHF), Hamburg.
- Rijks Museum voor Natuurlijke Historie (RMNH), Leiden.

VI. Publications:

Rutgers van der Loeff, M.M. & M.S.S. Lavaleye, 1986. Sediments, fauna and the dispersal of radionuclides at the N.E. Atlantic dumpsite for low-level radioactive waste. (Report of the Dutch DORA program).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-B-036-NL

Rijksinstitut voor Volksgezondheid
en Milieuhygiëne, RIVM
P.O. Box 1
NL-3720 BA Bilthoven

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M.J. Frissel
Laboratory for Radiation Research
R.I.V.M.
P.O. Box 1
NL-3720 BA Bilthoven

Telephone number: 030/74.25.15

Title of the research contract:

Countermeasures to the uptake of radionuclides from soils by food crops ; the long-term availability of radionuclides.

List of projects:

1. The long-term soil-to-plant transfer in the field, basic research long-term availability, and countermeasures to reduce soil-to-plant uptake with emphasis on practicability.

Title of the project no.:
Countermeasures to the uptake of radionuclides from soils by food crops; the long-term availability of radionuclides.
1. The long-term soil-to-plant transfer in the field, basic research long-term availability, and countermeasures to reduce soil-to-plant uptake with emphasis on practicability.

Head(s) of project:
J.F. Lembrechts

Scientific staff:

G.M.Desmet, M.J.Frissel, H.W.Köster and J.F.Lembrechts

I. Objectives of the project:

Earlier, long-term studies were made on the uptake of some important radionuclides by food crops. The soil-to-plant transfer factors proved to depend on the time elapsed between soil contamination and harvest and on the concentrations of organic matter in the soil. Present objectives:
1) Completion of the former study on the determination of transfer factors (1985 was the last year of a 4-year crop rotation scheme).
2) To study practical possibilities to decrease the uptake of radionuclides by different means, additions e.g. of organic matter and stable elements to the soil.
3) Specialized studies on uptake mechanism and bioavailability.

II. Objectives for the reporting period:

The lysimeter study on the impact of selected counter measures was extended. The counter measures (liming and addition of organic matter and stable isotopes) were not re-applied in order to investigate the persistence or delayed appearance of effects. Again no fertilizer was applied to those containers which remained unfertilized throughout 1986 and 1987. In order to allow a straightforward comparison with former results the production plan of the previous years was repeated.

In order to describe the relation between the composition of the soil liquid phase and the uptake of Sr-85 by plants a comparison was made between its uptake from nutrient solutions and from soils of which the interstitial liquid phase was studied as well.

III. Progress achieved:

METHODOLOGY

1. Experiments under natural conditions have been carried out on the 24 fields of the lysimeter, described in earlier reports. As in 1986 and 1987 beans and spinach were grown successively, on all containers. The concentration of Cs-134, Cs-137, Co-57, Co-60, Mn-54, Sr-85, Sr-90 and Zn-65 of dried plant and soil samples were measured. In May, June and September the interstitial soil solution was collected and analysed. Its pH, conductivity and the concentrations of several nutrients were determined.

A statistical analysis was made of the complete set of data, collected in 1986, 1987 and 1988.

2. The experimental techniques developed to study the bioavailability of Tc in soils and its accumulation by plants (progress report 1986) were used to extensively analyse the uptake of Sr. Both a centrifugal filtration method and an immiscible displacement method were used in order to isolate the soil liquid phase. The pot experiments under controlled conditions, started in 1987, were continued. Additional experiments were done on the effect of fertilization as well as on the influence of soil moisture content and liming upon the uptake of Sr. Lettuce plants (Lactuca sativa L. cv. Ysbergsla) were grown both on potted soil (about 1 kg/pot) and nutrient solutions (a modified and diluted Hoagland-Arnon nutrient solution). Conductivity, pH, Ca and Sr-85 content of the soil solution and the nutrient solution were measured and the Ca and Sr-85 content of the plant shoot.

RESULTS AND DISCUSSION

1A. Soil liquid phase -- The differences in chemical composition of the liquid phase of controle containers of the lysimeter as compared to specifically treated ones were of the same order of magnitude in 1988 as in 1987. The changes induced by the counter measures which were applied in the course of the previous year, thus persisted during the next growth period.

1B. Transfer factors (TF) -- In general the TFs, for all nuclides and both for spinach and beans, were higher in 1988 as compared to 1987 (10 - 60 %). The TFs of Sr-85 and Sr-90 (not reported on in 1987), increased throughout the three successive growth periods. The difference between the TF's of Cs-134 and those of Cs-137 persisted as well.

1C. Counter measures -- For most combinations of soil (N = 3), nuclide (N = 8) and plant (N = 2) the mean TF on containers treated with one of the counter measures was lower (up to 70 %) than the TF on controle containers. Changes were most apparent on sandy soils enriched with lime or organic matter. The effect of organic matter might in part be traced back to that of the simultaneously added neutralizing lime. The spread in the results obtained was, however, too large and the number of measurements still insufficient to draw hard conclusions, even though the various counter measures were

pronouncedly applied and often had clearly detectable effects on the soil solution composition.

2A. Observations on nutrient solutions -- Because of the mutual relationship between Ca and Sr, the uptake of Sr was studied in relation to the availability and uptake of Ca. Experiments with plants growing on nutrient solutions showed transfer of Sr-85 to be predominantly determined by the Ca/Sr ratio. Varying the concentration of other nutrients had minor effects. The uptake rate of Ca by lettuce was concentration independent within the concentration range studied. Actions and practices inducing comparable changes in both the Sr- and Ca-level of the soil solution will not change the Ca/Sr ratio and thus will have minor effects on the uptake rate. The TF was futhermore shown to decrease as a function of plant age.

2B. Observations on soils -- Addition of NPK-fertilizer and a decreasing moisture content of the soil not only augment the soil solution concentrations of both, Sr and Ca, but the nutritional status as a whole. Since the Ca/Sr ratio remained unchanged and increased levels of other nutrients cause only a slight decrease in Ca/Sr uptake, the observed changes in the Sr-85 content of the plant were rather limited.

The soil matrix and liming determine the ratio between Sr and Ca in the soil solution and consequently the transfer factor.

2. Conclusions -- As was observed two years ago for the accumulation of technetium by spinach, the uptake pattern of Sr-85 by lettuce from soils was consistent with that from hydroponic systems, i.e. : (1) transfer factors based on the concentration of the soil liquid phase (Bq per g fresh plant / Bq per ml soil solution) are of the order of magnitude of those measured on nutrient solutions, which roughly have a comparable chemical composition, and (2) in both systems changes in relevant variables induce comparable effects.

Since the interstitial soil liquid phase is concluded to mediate between the solid phase and the plant root, reliable interpretations of soil-to-plant transfer might as a rule be based on a separate study of the uptake from nutrient solutions on the one hand and of the effect of soil properties on availability on the other. This approach will allow a more basic evaluation of the processes affecting uptake kinetics and bioavailability.

IV. Objectives for the next reporting period:

a. For the last contract period a final series of field measurements concerning the effect of counter measures on radionuclide transfer is planned, in order to allow for an ameliorated final evaluation of the phenomena being studied. Attention will be particularly given to the uptake of Sr-90, a detectable fraction of which is present in the soil liquid phase.

b. The lab experiments on Sr will concentrate on some kinetic aspects of the interaction between solid and liquid phase when counter measures are applied. Some introductory experiments on the relation between the soil liquid concentration of Cs-134 and its uptake by plants will be done.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof.Dr.Ir. A. Cremers, K.U.L., Leuven, België
Prof.Dr. O. Vanderborght, U.I.A., Antwerpen, België

VI. Publications:

FRISSEL M.J., KEVERLING BUISMAN A.S., STOUTE J.R.D., MATTERN F.C.M. and DROST R.M.S. -- The availability of deposited Cs-137 to man. Proc. of the IVth Int. Symp. on Radioecology of Cadarache, 880314. Part II (1988), F 51-58

FRISSEL M.J. and KOSTER J. -- The IUR project on soil-to-plant transfer factors of radionuclides : expected values and uncertainties. In : Reliability of radioactive transfer models, G.M. Desmet, Ed. Elsevier, London (1988), 151-158

KOSTER H.W., KEEN A., PENNDERS R.M.J., BANNINK D.W. and DE WINKEL J.H. -- Linear regression models for the natural radioactivity (U-238, Th-232 and K-40) in Dutch soils : a key to anomalies. Radiation protection dosimetry. In press.

LEMBRECHTS J.F., VAN LOON L.R., VAN GINKEL J.H. and DESMET G.M. -- Interpretation of soil-to-plant transfer on the basis of soil solution chemical composition. Proc. of the IVth Int. Symp. on Radioecology of Cadarache, 880314. Part I (1988), D 169-178.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-B-198-P

Laboratorio Nacional de Engenharia
e Tecnologia Industrial (LNETI)
DPSR - Azinnhaga dos Lameiros
Estrada do Paço do Lumiar
P-1699 Lisboa

Head(s) of research team(s) [name(s) and address(es)]:

Dr J.P. Galvào
DPSR
LNETI
Estrada Nacional 10
P-2685 Sacavém

Telephone number: (1)255.49.81

Title of the research contract:

Behaviour of radionuclides and model development in aquatic ecosystems.

List of projects:

1. Radioecology of river ecosystems
2. Behaviour of radionuclides in the marine environment.

Title of the project no.: 1

Radioecology of River Ecosystems

Head(s) of project: M. Carolina Vaz Carreiro
L. Canelas, as leader of the contribution from DCEA/UNL

Scientific staff:
- M.C. Vaz Carreiro
- M.J. Madruga
- M.M. Sequeira
- J.A. Corisco
- L. Canelas
- A. Brogueira
- M.M. Brito

I. Objectives of the project:

The objective of this project is to obtain a better knowledge on the behaviour of radionuclides potentialy released into the river Tejo and on the modalities of transfer in the river ecosystems. It covers the radiological survey of the river and radioecological experiments on a simplified trophic chain from the Fratel dam, with the aim of establishing an experimental biological transfer model.

Implementation of a mathematical simulation model for predicting radionuclides dispersion and transport along river Tejo.

II. Objectives for the reporting period:

1. Prossecution of the physico-chemical characterization of the water, chemical characterization of the biota and sediments, and also the mineralogical characterization of these ones; qualitative knowledge of the phytocenoses and of the icthyofauna, and a lymnological study.
2. Experiments concerning the Co-60 transfer in a simplified trophic chain from Fratel dam and its behaviour in water-sediments interactions, were intended to begin.
3. First simulations for radioisotopes using the capacity of the installed model to simulate one non-conservative parameter. Implementation of a specific mathematical model for radionuclides simulation.

III. Progress achieved:

1. Methodology

1.1 Field study of River Tejo Radioecology at three points, Fratel dam, Barquinha and Valada

i) determination of the concentration of natural and artificial radionuclides in water, sediments and biota.

ii) determination of the physico-chemical quality of the river water.

iii) chemical and mineralogical characterization of the sediments.

iv) determination of trace elements concentrations by neutron activation analysis in water, sediments and biota.

v) lymnological study in order of the knowledge of the planktonic communities and of their trophic state.

vi) qualitative characterization of the river fauna and flora.

1.2 Radioecological experiments on a Trophic chain from the Fratel dam

i) study of radionuclides transfer in a freshwater trophic chain

ii) study of radionuclides behaviour in freshwater sediments

1.3 Modelling

Selection and implementation of the mathematical code, introducing in it the necessary modifications to fit the code to the specific hydrodynamic characteristics of river Tejo.

Field work for sampling and measuring local parameters over the river Tejo.

2. Results

2.1 Field study of River Tejo Radioecology

During this year the emphasis was placed on the following points:

The physico-chemical characterization of the water has been carried out seasonally, since the begining of 1987.

The determination of the major elements (Na, K, Ca and Mg) in river sediments and biota followed the same pattern.

The mineralogical characterization of the river sediments has been done only in summer and winter, also since 1987.

The determination of the trace elements concentrations by neutron activation analysis was performed only on the first samples of 1987 due to the shut down of LNETI's Research Reactor for maintenance; it will be on normal operation only by middle of 1989. However those first results were already object of several papers.

The lymnological study and the characterization of the phytocenoses were carried out during 1988, and the characterization of the fauna is still

being done.

The determinations of the natural and artificial radioactivity, as usually, have been carried out seasonally.

2.2 Radioecological experiments on a trophic chain from the Fratel dam

In what concerns the water-sediments interactions, the study with the ^{134}Cs was finished and a paper was presented at a Symposium.

A similar study with ^{60}Co has been initiated.

Concerning the ^{134}Cs transfer in a trophic chain of the Fratel dam, to finish the whole study 2 experiments were missing:

- the uptake of ^{134}Cs by Tinca tinca through the food, labelled Daphnia magna, which is already done;
- the retention of ^{134}Cs by tinca tinca following the ingestion of labelled D. magna, which is still running.

2.3 Modelling

A lot of parameters of river Tejo were collected and analysed for an increased knowledge about its hydrologic and hydraulic characteristics.

A complementary work carried out in the laboratory, fitting and running the models, and on field, gathering data for the hydraulic and hydrodynamic characterisation of the river, was done.

Modifications were done on the mathematical algorithm to extend its application to three major dams in Tejo basin.

3. Discussion

Data obtained until now seems to point out that the river waters, in the three places under study, may be considered as mesotrophic; the nutrient salts along the year are not high and the density and biomass of phyto and zooplankton are not high as well, but more data is necessary to know whether it is confirmed or not. The results obtained in the radiological survey, during 1988, are within the usual range.

Concerning the study on the behaviour of ^{60}Co in freshwater sediments from Fratel dam, certain difficulties were found in the determination of the physico-chemical forms of cobalt, which are expected to be solved during the next period of the project.

Concerning modelling the planned objectives for the period were generally accomplished.

IV. Objectives for the next reporting period:

The prossecution of the radiological survey of Tejo river and of the characterization of the ecosystems at the three stations.

To perform the experiments concerning the ^{60}Co transfer through a simplified trophic chain from Fratel dam and the interaction water-sediments from Fratel too.

Implementation of the model to predict radionuclides concentration and the linking with the hydrodynamic one.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

- Experimental Study of Cs-134 behaviour in freshwater sediments
  M. José Madruga, M. Carolina Vaz Carreiro, Antônio O. Bettencourt
  IV International Symposium of Radioecology
  Cadarache, France, 14-19 March 1988.

- Análise Multielementar de Peixes e Água do Rio Tejo pela Técnica de Análise por Activação com Neutrões.
  M. Fátima Reis, M. Carmo Freitas, M. Carolina Vaz Carreiro, Eduardo Martinho
  1ª Conferência Nacional sobre a "Qualidade do Ambiente",
  Aveiro,Portugal, 22-24 Febru. 1988.

- Determination of the Level of some Heavy Metals in an Aquatic Ecosystem by Instrumental Neutron Activation Analysis
  M.C. Freitas, M.C. Vaz Carreiro, M.F. Reis, E. Martinho
  Environmental Technology Letters, vol. 9, p.p. 969-976, 1988.

- Study of the Pollution by Trace Metals in Ecosystems of the Tagus River by Instrumental Neutron Activation Analysis.
M.C. Vaz Carreiro, M.F. Reis, M.C. Freitas, E. Martinho
to be presented at the 9th International Symposium on Environmental Pollution, Toronto, Canada, 8-9 June 1989.

- Controlo Radiológico do Rio Tejo: 1981 a 1986
M.M. Sequeira, M.C. Vaz Carreiro, A.O. Bettencourt
LNETI/DPSR - B Nº 106 (1988)

- Controlo Radiológico do Rio Tejo: 1987-1988
M.M. Sequeira, M.C. Vaz Carreiro, A.O. Bettencourt
under publication.

Title of the project no.: 2

Behaviour of Radionuclides in the Marine Environment

Head(s) of project: A.O. Bettencourt

Scientific staff: A.O. Bettencourt
M.D.T. Elias
F.P. Carvalho
G.C. Ferrador

I. Objectives of the project:

To study the distribution and behaviour of the more significant natural and artificial alpha-emitters in the marine environment and their respective contribution to the radiation doses to humans and to marine organisms.

To evaluate field concentration factors of artificial and natural alpha--emitters in deep-sea fish and common sea-food from coastal areas, significant in the population diet.

II. Objectives for the reporting period:

Enlargement of ^{210}Po - ^{210}Pb analysis to deep sea fauna and transfer along food chains. Comparison of ^{210}Po concentration in marine and terrestrial foodstuffs.

Continuation of analyses of ^{239}Pu and ^{241}Am in marine biota and starting of their measurements in sea-water.

III. Progress achieved:

1. Methodology

Analysis of ^{210}Po are performed through the classical procedure of sponteanous plating of ^{210}Po together with ^{209}Po isotopic tracer onto a silver disk from hydrocloric solution. A second ^{210}Pb plating a few months later enables the calculation of the ^{210}Pb activity in the sample. Measurements are made by alpha spectrometry with silicon surface barrier detectors.

The transuranics are analysed by ion exchange chromatography (AG 1 X 8), solvent extraction with HDEPH and electroplating. Measurements are also made by α spectrometry.

2. Results

The analysis of ^{210}Po and ^{210}Pb, formerly focussed on coastal organisms, were enlarged during 1988 to include marine fauna from the NE Atlantic gyre. Sampling of marine biota from several depths in the water column and from the abyssal floor was possible through the kind cooperation of a FRG oceanographic cruise to the NEA dumping area.

^{210}Po measurements in zooplankton and large pelagic crustaceans confirms the existence of very high concentrations in these biota.Specially the crustacean hepatopancreas display high concentrations, ranging between 1 and 39 $Bq.g^{-1}$ (wet wt) in sergestid prawns, while the muscle tissue display 0.09 - 1 $Bq.g^{-1}$.

Myctophid fishes (Myctophum, Electrona, Ceratoscopelus), from the mesoplagic zone of the water column, display ^{210}Po concentrations in muscle tissue ranging from 0.1 through 7.5 $Bq.g^{-1}$. In the same oceanic layer, the common hatchet fish Argyropelecus display the highest whole-body Po concentration ever reported for a biological species: 29.6 $Bq.g^{-1}$. Fish from the lower mesoplagic zone, as Stomiidae and Chauliodontidae, display 0.07-0.16 $Bq.g^{-1}$ for muscle tissue. Typical inhabitants of the abyssopelagic zone, as Melamphaidae and the gulper-eel Eurypharynx display concentrations about 0.08-1.15 in muscle tissue.

Abyssobenthic fishes, as macrourids and brotulids, display ^{210}Po concentration of 0.006 $Bq.g^{-1}$ and 0.27 $Bq.g^{-1}$ in muscle tissue, respectively. These fishes live in close vicinity of the ocean floor and feed upon benthic invertebrates as echinoderms (0.3-3 $Bq.g^{-1}$), molluscs (6.7 $Bq.g^{-1}$) and ascidians (1-2.5$Bq.g^{-1}$). Analysis of other naturally occurring α-emitters are now being started on

coastal samples, for comparison purposes on radionuclide concentrations and radiation dose regime experienced by marine fauna.

^{210}Po and ^{210}Pb measurements in samples from the terrestrial environment, namely meat, vegetables, fruits, milk, eggs, and wine, indicate much lower concentrations of those nuclides. Fresh fruits and vegetables display ^{210}Po concentrations between 0.01-0.8 $mBq.g^{-1}$. ^{210}Po concentration in meat varies between 0.05 and 0.3 $mBq.g^{-1}$, and similar concentrations were found in milk, wine, beer and eggs. Higher concentrations were measured in certain samples as mushrooms, 3 $mBq.g^{-1}$, and wild birds flesh, 23 $mBq.g^{-1}$.

Fishes collected at Madeira and at Sesimbra (portuguese continental coast) were analysed for $^{239+240}Pu$ and ^{241}Am. The analyses were performed on muscle samples and also on some other organs.

The concentrations in muscle of deep sea pelagic fish (Aphanopus carbo - 1200m, Alepocephalus bairdii - 1600m and Lepidon eques -1150m) range from $\leq$ 0.08 to 0.28 mBq/Kg for $^{239+240}Pu$ and from 0.15 to 0.39 mBq/Kg for ^{241}Am.

The concentrations in muscle of fishes living at depths down to 600m (Thunnus alalunga, Makaira nigricans, Thunnus obesus and Sphyraena sphyraena) are of the order of 0.20 mBq/Kg both for $^{239+240}Pu$ and ^{241}Am (with an isolated value of 1.4).

In other organs (liver and gonads) and remainders the analyses are performed in a litttle amount of ashes and the obtained values were almost always below the detection limit (of the order of 1.5 mBq/Kg).

3. Discussion

Previous work had firmely stated ^{210}Po as the main internal α-emitter in marine organisms. Radiation doses from ^{210}Po alone vary widely, accordingly to its concentration, nevertheless those doses are much higher than the ones due to other natural and man-made radionuclides. ^{210}Po concentration in oceanic fishes also spread over a wide range, larger than found in coastal biota. Again ^{210}Po concentration is not related with depth in ocean, but there are indications that it correlates with the trophic levels and, thus, concentrations are strongly dependent upon the food-chain transfer in each particular environment.

Further work seems advisable to clearly delineate the dose regimes in deep sea fauna and to establish food-chain transfer factors.

From the existing data base on ^{210}Po, it seems clear that the marine foods, in which concentrations are 10^3 higher than in terrestrial foods, will make a major route for this nuclide transfer to humans. Other pathways, as inhalation and drinking water, will be adressed in further analysis. The progress achieved may be considered consistent with the objectives planned for the reporting period. Analysis of U, Th, Ra isotopes in marine biota are now being started.

IV. Objectives for the next reporting period:

Analysis of U, Th, Ra isotopes in marine biota.

A comprehensive review of data on these nuclides and radiation dose regimes to marine organismes.

Transfer of natural α-emitters to man through the food chain.

Analysis of transuranics in water for the calculation factors. Assessment of doses due to the artificial alpha emitters and their comparison with those due to the natural ones.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

F.P. Carvalho (in press). Polonium-210 in marine organisms: a wide range of natural radiation dose domains.

IV Int. Symp. on the Natural Radiation Environment, CEC-DOE, Lisbon 7-12 Dec. 1987.

F. P. Carvalho. A sequential extraction technique for ^{210}Pb, ^{210}Bi, ^{210}Po analysis in environmental samples (in Portuguese).

VI Conferência Nacional de Física, 26-29 Sept. 1988, Aveiro, Portugal.

F.P. Carvalho. Polonium-210 in marine fish. Rapp. Comm. Int. Mer Médit. 31(2): 246 (1988)

A.O. Bettencourt, M.D.T. Elias, G. Ferrador. Vigilância radiológica do meio marinho relacionada com a imersão de resíduos radioactivos no Oceano Atlântico Nordeste.

2º Congresso Geral de Energia Nuclear, 23-26 Apr. 88., Rio de Janeiro, Brasil

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Contract no.: BI6-B-037-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr A. Grauby
SERE-DERS
CEA-CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Telephone number: 42/25.73.25

Title of the research contract:

Behaviour of radionuclides in marine, freshwater and terrestrial environments.

List of projects:

1. Behaviour of Neptunium in marine ecosystem
2. Exchange of radionuclides between sea and atmosphere
3. Radioecology of continental waters
4. Radium transfer in fresh water ecosystem
5. Cycling of tritium
6. Radiological impact of radionuclides accidentally released
7. Comparative study of soil to plant transfer of Np, Pu and Am
8. Radionuclides deposition on vegetation and soils

Title of the project no.: 1

BEHAVIOUR OF NEPTUNIUM IN MARINE ECOSYSTEM

Head(s) of project:

Dr GRAUBY

Dr GUEGUENIAT

Scientific staff:

P. GERMAIN, R. GANDON, Th. LEROY

I. Objectives of the project:

Les données relatives aux transferts du neptunium vers les sédiments et les organismes aquatiques sont très rares. Or quelques travaux montrent la présence de 237-Np dans l'environnement marin. Puis 237-Np sera l'un des constituants majeurs, à long terme, des déchets éventuellement stockés en mer. De plus, par certains aspects physicochimiques, Np s'écarte des autres transuraniens, ce qui implique à son sujet des études spécifiques. Aussi, il est nécessaire de développer la connaissance des transferts (physicochimie, définitions des voies de transfert, cinétiques de fixation et de perte,organotropisme, définition de bioindicateurs, détermination des FC et kd...) dans le milieu marin. Les études sont menées selon deux approches : l'une in situ, l'autre expérimentale.

II. Objectives for the reporting period:

En 1988, les études ont porté sur le transfert du neptunium chez des mollusques suspensivores, brouteurs et carnivores à partir d'études in situ et expérimentales (utilisation des isotopes 237 et 239 Np).
Les points principaux d'études portent sur les cinétiques de fixation et de perte chez les coques, moules et nucelles , puis sur l'organotropisme du neptunium chez les moules, les nucelles et les gibbules. Enfin une discussion est engagée sur l'incidence des protocoles expérimentaux sur les résultats et sur la "voie métabolique" du neptunium chez les mollusques.

III. Progress achieved:

En 1988, les études ont porté sur le transfert du neptunium chez des mollusques suspensivores, brouteurs et carnivores, à partir d'études in situ et expérimentales (transfert à partir de l'eau de mer en utilisant les isotopes 237 Np et 239 Np, placés à l'état NpO_2^+).

Cinétiques de fixation et de perte

Une première étude expérimentale du transfert du Np à partir de l'eau de mer a précédemment indiqué pour deux espèces suspensivores, coques et moules, des facteurs de concentration pour les chairs de coques de 2 à 10 et de 40 à 80 pour les coquilles, et pour les chairs de moules de 2 à 7 et de 20 à 50 pour les coquilles. Dans les deux cas, après trente jours de contamination l'intensité de la fixation faiblit mais cependant se poursuit. Ces études ont été réalisées selon le protocole décrit dans (1).

Des moules en provenance de la côte Est du Cotentin ont été introduites en rade de Cherbourg afin de suivre in situ la cinétique de fixation du 237 Np (les dosages ont été effectués en utilisant l'analyse par activation neutronique). Les résultats sont les suivants, en mBq kg^{-1} frais : T0 < 0,20 ; T = 29 jours : 0,06 + 0,01 ; T = 57 jours : 0,10 + 0,02 ; T = 85 jours : 0,19 + 0,04 ; T = 197 jours : 0,09 + 0,02 ; T = 302 jours : 0,29 + 0,06. Les niveaux de 237 Np augmentent durant les 3 premiers mois de l'expérience. Ensuite ces fluctuations apparaissent, qui sont, sans doute, à relier aux évolutions des rejets de l'usine de La Hague. Ces conclusions rejoignent celles concernant une étude des cinétiques de fixation in situ d'émetteurs γ par des moules (2). Une nette différence apparaît entre le taux de 237 Np enregistré dans les chairs de moules en 1980 à 5 km de l'émissaire de l'usine de La Hague, 2 mBq kg^{-1} frais (2), et celui enregistré en rade de Cherbourg à 30 km de l'émissaire, 0,29 mBq kg^{-1} frais.

L'élimination du 237 Np a été étudiée chez les moules en laboratoire. Deux composantes ont été observées dans les chairs. Après 21 jours il persiste 34 % de l'activité initiale et la période biologique est de l'ordre de 20 jours. La cinétique d'élimination est plus rapide que celle enregistrée en laboratoire par Guary et Fowler (3) chez Mytilus galloprovincialis. Les raisons de ces différences ne sont pas connues. Il est à noter que ces auteurs ont observé une perte plus rapide in situ (Tb 1/2 331 jours), qu'en laboratoire. De son côté Dahlgaard expérimentant différemment a calculé des périodes biologiques pour Mytilus edulis variant de 53 à 300 jours (4). La perte de 237 Np a également été étudiée chez le mollusque gastéropode carnivore Nucella lapillus selon des conditions expérimentales identiques à celles des moules. Après 50 jours il reste encore près de 20 % de l'activité initiale tant dans les chairs que dans les coquilles. Par contre, au début, la perte est rapide dans la chair puisqu'après 5 jours il ne persiste que 35 % de l'activité initiale contre 67 % dans les coquilles. Quant à la phase fixation, l'évolution est lente après une quinzaine de jours de contamination, comme l'indiquent les facteurs de concentration ci-après :

	chairs	coquilles
T 4 jours	1,1	15,5
T 9 "	1,2	29,9
T 16 "	5,4	31,1
T 23 "	8,0	31,3
T 30 "	5,0	42,4

Ces valeurs sont notablement inférieures à celles obtenues in situ à Goury en janvier 1986 (46 à 118 pour les chairs, 100 à 255 pour les coquilles (5).

Organotropisme

Les résultats de l'expérience précédemment citée indiquent pour les coquilles des nucelles un pouvoir de fixation du 237 Np supérieur à celui des chairs, confirmant ainsi les derniers résultats obtenus in situ (5) mais infirmant les premiers résultats in situ obtenus dans notre laboratoire (1).

L'isotope 239 Np a été utilisé, selon le protocole décrit dans (6), pour étudier plus facilement la distribution du Np dans les organes des parties molles de mollusques après quelques jours de contamination par l'eau de mer. Avec les nucelles, une première expérience montre une potentialité de fixation de l'appareil digestif supérieure à celle du muscle (950 cpm g^{-1} frais contre 400 cpm g^{-1} frais) ; une seconde expérience atténue cette différence (3400 cpm g^{-1} frais pour l'appareil digestif et 3600 cpm g^{-1} frais pour le muscle.
Chez le gastéropode brouteur Gibbula umbilicalis, l'appareil digestif fixe nettement plus le Np que les muscles (81000 cpm g^{-1} frais contre 18000 cpm g^{-1} frais).
Chez Mytilus edulis la masse viscérale fixe après 14 jours d'accumulation, 82 % du Np contenu dans l'ensemble des chairs, les branchies 2,5 %, le manteau 12 %, le muscle 2,7 %, le pied 0,5 %, les palpes labiaux 0,3 %. Dans toutes ces expériences avec le 239 Np, les coquilles ont montré un pouvoir de fixation supérieur aux chairs.

Discussion

Des décalages relativement importants apparaissent entre les faits observés in situ et au laboratoire, et dans ce dernier cas, entre les diverses modalités expérimentales. En ce qui concerne ce dernier point nous avons réalisé les expériences suivantes : contamination d'un lot A de moules avec du 239 Np (modalités identiques à celles décrites dans (6)) ; après 24 heures de contact, l'eau de mer est utilisée pour marquer un lot B de moules, le lot A étant à nouveau contaminé par de l'eau de mer fraîche et ainsi de suite. Le tableau suivant donne l'évolution des rapports cpm g^{-1} frais coquille/cpm g^{-1} frais chair :

Rapport d'activité coquille/chair. Mytilus edulis

	^{239}Np		^{237}Np (2)
Jours	Série A	Série B	
2	0,83		
3		1,9	
4	0,71		
5		2,9	
6	0,90		
7		1,6	
8	0,68		10,4
9		1,9	
10	0,61		
11		3,1	18,9
12	0,97		
13		1,3	
15	0,82		
16			11,8
21			6,2

Une étude physico-chimique du 239 Np dans l'eau de mer ne montre pas d'évolution de cet élément à T = 0 jour, 2 jours et 5 jours de contact eau de mer - moules (protocole conforme à celui décrit dans (6) : passage sur résine d'eau de mer filtrée marquée par 239 Np, puis élutions avec de l'eau distillée et de l'eau de mer). Il faut donc chercher une autre cause à la différence observée chez ces rapports d'activité : série A (changement d'eau toutes les 24 h), rapport inférieur à 1 ; série B (eau de mer en contact 48 h avec les moules) rapport supérieur à 1, tendance identique à celle de l'expérience menée avec 237 Np où le changement d'eau était réalisé toutes les 48 h.
Les mucopolysaccharides présents dans le tractus digestif expliquent peut être cette différence. Ils fixeraient le neptunium dans un milieu "frais" et participeraient fortement à la teneur du Np dans les chairs. Puis ils seraient éliminés, d'où une teneur moindre dans les chairs au delà de 24 h. De toute façon, de l'ensemble de ces études, il se confirme l'importance des structures calcifiées dans la fixation du Np. Cette importance est cependant atténuée

chez Gibbula umbilicalis et surtout Patella sp. d'après les résultats récents in situ (5). De plus, l'appareil digestif montre un fort pouvoir de rétention par rapport aux autres tissus mous et on peut envisager que le neptunium absorbé avec l'eau de mer suit chez les mollusques étudiés les voies du métabolisme digestif. Cependant un transfert du neptunium vers la glande digestive à partir de la branchie ne peut pas être écarté.

Références

(1) J. Environ. Radioactivity, 5 (1987) 319-331.
(2) R. CEA-5211 (1983).
(3) Mar. Sci. Comm. (1977) 211-229.
(4) Mar Ecol. Prog. Ser. (1986) 157-165.
(5) Neptunium-237 in the marine environment. Determination in animal and plant species in the english Channel : biological indicators and trophic relationships. En préparation.
(6) J. Environ. Radioactivity 5 (1987) 37-55.

IV. Objectives for the next reporting period:

A partir d'espèces indicatrices de mollusques nous étudierons la dispersion du 237 Np autour du Cotentin. Puis l'accent sera porté sur les transferts du neptunium vers des espèces de crustacés et de poissons. Enfin après cinq ans d'études nous comparerons le comportement du neptunium avec les autres éléments transuraniens, plutonium, américium, curium.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs Engelmann et Pinte - C.E.A. C.N.R.S Laboratoire d'analyse par activation Pierre Süe, CEN-Saclay, 91191 GIF-sur-Yvette Cedex, France.

VI. Publications:

P. Germain et P. Pinte, 1988. Neptunium 237 in the marine environment. Determination in animal and plant species in the english channel : biological indicators and trophic relationships. Second international conference on low level measurements of actinides and long-lived radionuclides in biological and environmental samples, Akita City, Japan, May 16-20, 1988.

Title of project no.: 2

Echange de radionucléides entre la surface de la mer et l'atmosphère : étude expérimentale des mécanismes de transfert

Head(s) of project

C. CAPUT

Scientific staff:

D. GAUTHIER
Y. BELOT

I. Objectives of the project:

Etude en laboratoire des mécanismes par lesquels une partie des radionucléides artificiels contenus dans la mer est mise en suspension dans l'atmosphère. On étudiera le rôle de la matière particulaire contenue dans l'eau de mer, le rôle des enduits organiques et des caractéristiques du bullage.

II. Objectives for the reporting period:

L'objectif poursuivi est d'étudier les mécanismes par lesquels les particules contenues dans l'eau de mer se concentrent dans les embruns mis en suspension dans l'atmosphère. Pour cela nous avons mis en oeuvre une méthode de simulation expérimentale, qui consiste à remplacer les particules naturelles de l'eau de mer par des particules artificielles fluorescentes monodispersées. Les expériences réalisées ont eu pour objectif d'étudier l'influence de la taille et de la concentration des particules sur l'efficacité de leur mise en suspension dans l'atmosphère.

III. Progress achieved

Introduction et rappel

Au cours de 1988 nous avons étudié les facteurs qui influencent la remise en suspension des particules contenues dans l'eau de mer. Pour cela nous avons utilisé une méthode de simulation qui a été décrite en détail dans le compte-rendu de l'année précédente. De manière résumée, cette méthode comporte les étapes suivantes:

1) Une suspension de particules dans l'eau de mer est préparée en ajoutant à un échantillon d'eau de mer une petite quantité (1 à 10 mg) de particules fluorescentes monodispersées enduites d'une couche d'albumine, de taille comprise entre 0,1 et 6,8 µm. Les particules contenues dans la suspension ainsi préparée ne coagulent pas et restent par conséquent parfaitement monodispersées.

2) Cette suspension est introduite dans un dispositif de bullage précédemment mis au point. Les bulles produites par passage d'air à travers une membrane poreuse hydrophobique montent à travers la suspension, éclatent en surface en produisant un embrun qui est collecté sur un impacteur d'Andersen à 8 étages ou sur un dispostif simplifié qui collecte toutes les particules de diamètre inférieur à 9 µm.

3) L'embrun ainsi collecté est analysé de façon à déterminer son contenu en sodium et en particules fluorescentes.Le sodium est mesuré par potentiométrie au moyen d'une électrode spécifique et les particules fluorescente au moyen d'un fluorimètre. Le facteur de transfert (TF) de chaque substance (particules ou sodium) est la fraction transferée à l'atmosphère par unité de temps pendant la durée de l'expérience de bullage. Le facteur de transfert des particules étant généralement beaucoup plus élevé que le facteur de transfert du sodium, le rapport de l'un à l'autre est appelé facteur d'enrichissement (EF).

Résultats obtenus en 1988

Des essais ont été effectués pour étudier l'influence de divers paramètres sur le transfert des particules entre l'eau de mer et l'atmosphère. Les paramètres principaux étudiés sont la taille et la concentration des particules en suspension dans l'eau de mer. Les essais de bullage ont été effectués avec des suspensions contenant des particules de taille comprise entre 0.1 µm et 6.8 µm, et de concentrations dans l'eau de mer égales à 1 ou 10 mg/l . Les facteurs d'enrichissement étant sensiblement indépendant de la taille des embruns, nous avons déterminé les facteurs d'enrichissement globaux qui correspondent à la totalité

des embruns de taille inférieure à 9 µm. Nous donnons sur la figure 1 les résultats obtenus pour une couche d'eau de 5 cm d'épaisseur et un débit de bullage de 1.41 m^3/h par m^2 de surface d'eau.

Il apparaît que le facteur d'enrichissement augmente lorsque la taille des particules et leur concentration dans l'eau de mer diminuent. Le facteur maximum obtenu jusqu'à présent est de 2,5 10^3 pour des particules de 0,1 à 1 µm et une concentration de 1 mg/L. Il n'est plus que de 1,5 10^2 pour des particules de 6,8 µm et une concentration de 10 mg/L.

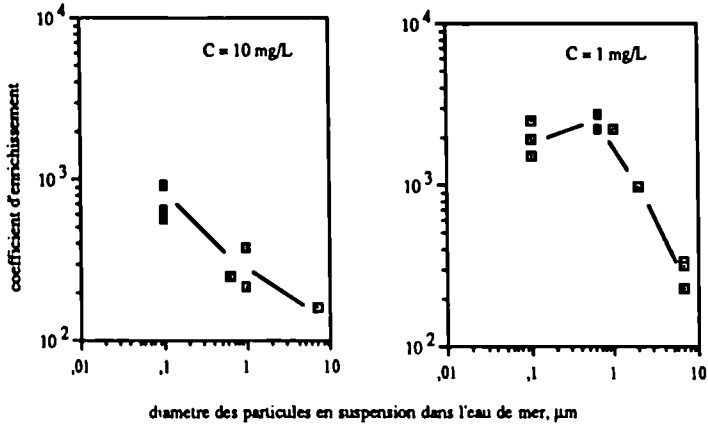


Fig. 1: Facteur d'enrichissement des embruns en fonction du diamètre des particules en suspension pour deux concentrations de particules (10 mg/L et 1 mg/L)

Dans quelques essais, les embruns ont été séparés en classes granulométriques au moyen de l'impacteur d'Andersen. Il apparaît que l'enrichissement des gouttelettes d'embruns est le même quelle que soit la taille des gouttelettes.

Conclusions provisoires

L'enrichissement des embruns en radionucléides transuraniens correspond en fait à leur enrichissement en matière particulaire. Cet enrichissement est d'autant plus important que les particules contenues dans l'eau de mer sont plus petites et que leur concentration est plus faible. Ces particules sont captées par les bulles qui s'élèvent dans la colonne d'eau, puis sont incorporées dans les gouttelettes (film drops) qui résultent de l'éclatement des bulles.

IV. Objectives of the next reporting period

Des expérimentations complémentaires seront réalisées en 1989 pour étudier plus particulièrement le mécanismes par lequel les bulles captent les particules en s'élevant dans la couche d'eau traversée. On s'attachera particulièrement à déterminer le facteur d'enrichissement en fonction de l'épaisseur de la couche d'eau traversée. Une publication de l'ensemble des résultats est en cours de préparation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]

VI. Publications

Title of the project no.: 3 **RADIOECOLOGY OF CONTINENTAL WATERS**

Etude comparée des eaux continentales du bassin de la Meuse et du bassin du Rhône.

Head(s) of project:

FOULQUIER, L ; GRAUBY, A

Scientific staff: **LAMBRECHTS, A ; BAUDIN, J.P ; CHARMASSON, S ; CALMET, D ; GONTIER, G ; NUCHO, R ; REMILLET, J.N ; DIMEGLIO, Y.**

I. Objectives of the project:

- Etudier la radioécologie du bassin Rhôdanien et de son débouché en méditerranée par des prélèvements réguliers d'eau, de sédiments, de végétaux aquatiques et de poissons.
- Evaluer l'impact des installations nucléaires.
- Etudier les mécanismes de transfert, du 60 - Co dans une chaîne trophique expérimentale d'eau douce - Modéliser ces transferts afin de pouvoir interpréter ces résultats obtenus "in situ".

II. Objectives for the reporting period:

- Poursuivre les campagnes de prélèvements sur le Rhône et en bord de mer afin d'établir un bilan de l'impact de Tchernobyl comparé à celui des installations nucléaires.
- Faire quelques mesures de tritium car les résultats obtenus soulèvent des questions en fonction du terme source.
- Les travaux concernant le transfert du ^{60} Co à une algue planctonique (Scenedesmus obliquus) ont porté sur l'influence de la concentration du milieu en cobalt stable et sur la rétention du radionucléide par des cellules placées en décontamination.
- L'étude de la bioaccumulation par la carpe du ^{60} Co contenu dans 3 types de nourriture a été menée à son terme.

III. Progress achieved:

ETUDE DU BASSIN RHODANIEN

- Etude de terrain

Les mesures effectuées en 1987 ont été analysées. Les radionucléides provenant des retombées de l'accident de Tchernobyl, qui avaient masqué en 1986 l'influence des effluents liquides des installations nucléaires, ont progressivement décru. Le Ru-103 a complètement disparu des compartiments du fleuve. Le Ru+Rh-106 n'est plus détectable chez les poissons, il est inférieur aux limites de détection chez les végétaux aquatiques. Les évaluations des périodes biologiques sont résumées dans le tableau suivant :

Périodes biologiques des 4 principaux radionucléides présents dans les retombées de Tchernobyl (les périodes sont exprimées en jours avec entre parenthèses le % de radioactivité éliminé).

		Cs134		Cs137		Ru103		Ru+Rh106	
poissons	Tb1	10	(48%)	10	(43%)				
	Tb2	346	(51%)	346	(56%)				
bryophytes	Tb	6	(46%)	6	(46%)	12	(58%)	4	(82%)
	Tb2	99	(53%)	115	(53%)	63	(42%)	173	(18%)
phanéroga-	Tb1	23	(99%)	21	(85%)	23	(99%)	18	(76%)
mes immergées	Tb2	693	(1%)	693	(15%)	63	(1%)	77	(23%)

La radioactivité artificielle du fleuve revient progressivement au niveau qu'elle avait avant mai 1986. Elle est de 25 à 50 fois inférieure à la radioactivité naturelle dans les zones soumises aux effluents des centrales et du même ordre de grandeur en aval de l'usine de Marcoule.

Les échantillons prélevés en 1988, dans toutes les portions du Rhône, ont été conditionnés et adressés au laboratoire de métrologie radioactive. Les résultats ne nous sont pas encore revenus.

Quelques mesures de tritium ont été effectuées sur des échantillons représentatifs du Rhône. Les valeurs trouvées dans le Léman sont relativement faibles (500 Bq/l d'eau de combustion pour les sédiments, inférieurs à 50 pour les végétaux, de 12 à 30 pour les poissons).

A l'inverse dans le fleuve, les valeurs sont beaucoup plus élevées et très hétérogènes (y compris dans la zone située en amont de toutes les installations nucléaires) ne permettant pas, pour l'instant de montrer clairement l'impact des différents termes sources. Une étude s'impose pour comprendre l'origine et le devenir du tritium dans le Rhône. Le facteur de concentration du H3 dans les poissons est supérieur à 1 ce qui va à l'encontre des idées généralement admises.

- Etude de laboratoire

L'influence de la présence de cobalt stable sur l'accumulation du 60 Co par Scenedesmus obliquus a été étudiée en considérant 5 concentrations de l'élément. Ces concentrations, 0, 0,3, 2, 7 et 15µg/l, correspondent à des valeurs rencontrées in situ.

Les valeurs de tous les paramètres radioécologiques démontrent très clairement que l'importance de la fixation du 60Co par les algues est inversement proportionnelle à la concentration de l'isotope stable dans le milieu. A cet égard les écarts constatés pour le facteur de concentration sont particulièrement significatifs. Ainsi les valeurs maximales sont de 24 000, 22 000, 18 500, 8 000 et 5 000 respectivement pour les concentrations en cobalt stable de 0, 0,3, 2, 7 et 15µg/l. Comme le montre la figure 1 une relation linéaire peut être établie entre le facteur de concentration du 60Co (FC) et la concentration en élément stable du milieu (C).

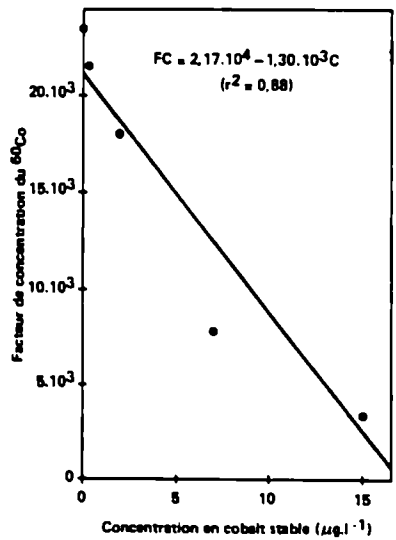


Figure 1 : relation entre le FC du 60Co par Scenedesmus obliquus et la concentration du cobalt stable dans le milieu

La rétention du 60Co par Scenedesmus obliquus a été étudiée pour diverses conditions expérimentales pouvant correspondre à des situations rencontrées in situ ou représentant les différentes étapes du cycle de développement d'une population algale. Parallélement à des études menées avec des algues placées dans des conditions normales de culture, la désorption a été suivie sur des cellules tuées après la phase de fixation du radionucléide. La durée de la contamination préalable a été prise en compte ainsi que la présence de chélatant dans le milieu de décontamination (EDTA et ClNa).

L'élimination du ^{60}Co par scenedesmus obliquus est un processus très rapide, quel que soit le temps de contamination préalable. La cinétique de la décroissance de la radioactivité met en évidence le caractère particulièrement labile de la liaison entre le radionucléide et les algues. D'autre part l'absence d'influence de l'obscurité et de la mort des cellules démontre que la perte du ^{60}Co est due à un phénomène essentiellement passif.

L'influence de la durée de la phase d'accumulation sur l'élimination de la fraction faiblement fixée du radiocobalt est très peu marquée. Par contre, la période biologique longue ainsi que le taux final de rétention du ^{60}Co augmentent correlativement à l'allongement du temps de contaminaiton. Ces données traduisent une augmentation de la solidité de la liaison entre les algues et le radionucléide. Elles peuvent être interprétées comme le résultat d'une pénétration de l'élément à l'intérieur des cellules, dont l'importance serait proportionnelle au temps de contact. La contaminaiton de Scenedesmus obliquus peut donc être attribuée à une adsorption du ^{60}Co, dont la contribution diminuerait progressivement , à laquelle s'ajouterait une accumulation intracellulaire croissante du radionucléide. L'évolution de l'importance relative de ces deux phénomènes est conforme aux résultats concernant la décontamination des algues en présence d'EDTA qui montrent que la désoption du ^{60}Co la plus forte se produit pour les cellules des cultures maintenues le moins longtemps en contamination.

- Etude en méditerranée

Les missions de prélèvements d'échantillons se sont réalisées mensuellement sur 7 stations de la zone d'expansion du Rhône (Port Camargue à Cap Couronne). Elles ont été complétées par des études spécifiques des zones mytilicoles du golfe de Fos et 7 campagnes océanographiques sur l'ensemble du littoral méditerranéen français. Ces dernières ont permis la collecte de plusieurs centaines d'échantillons, sur le littoral corse (09/85, 09/86 et 07/87), l'ensemble du littoral de Banyuls à Menton (04/86 et 11/86), et, de façon plus focalisée, sur le proche delta rhodanien (02/86, 09/87).

L'ensemble des organismes marins prélevé le long du littoral méditerranéen, en dehors de l'aire d'épendage rhodanienne, pendant les mois précédents la date de l'accident de Tchernobyl, est caractérisé par l'absence de tout radioélément émetteur gamma, hormis le 137 Cs, quelquefois détecté à de très faibles teneurs.

Sur les stations proches de l'embouchure (entre Port Camargue et Ponteau), on observe une relation entre les niveaux d'activité et la distance à l'embouchure, avec des teneurs plus élevées sur le côté ouest, soumis à l'influence du courant liguro-provençal.

A la suite de l'accident de Tchernobyl, les organismes marins répondent très rapidement à l'injection des radioéléments caractéristiques de cet accident puis les niveaux diminuent rapidement durant les mois suivants l'accident, traduisant ainsi la disparition de ces éléments au sein des masses d'eau.

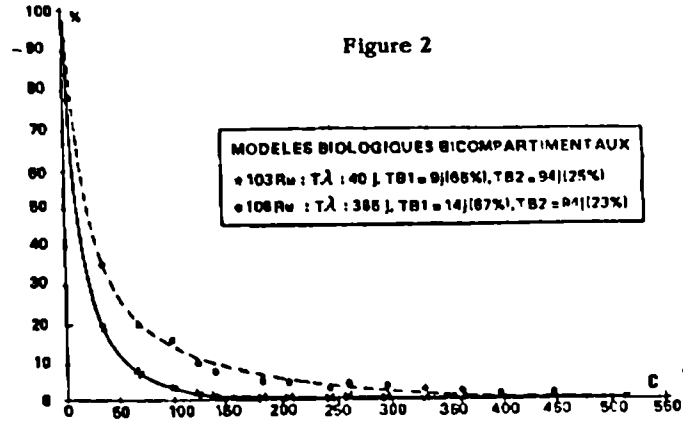
A la zone sous l'influence du Rhône, deux autres zones reflètent cette injection : la partie Est du littoral, avec un gradient décroissant de Menton à Toulon et la côte Est du littoral Corse. On remarque dans ces zones, l'apparition d'émetteurs gamma non observés jusqu'alors ou présents très occasionnellement (tableau).

	^{103}Ru	^{106}Ru	^{110m}Ag	^{134}Cs	^{137}Cs	^{144}Ce	^{141}Ce	^{131}I
CHLOROPHYCEAE Codium dichotoma	440.0±20	180.0±20	22.0±3	10.0±3	19.0±3	42.0±4	31.0±3	1150.0±130
PHAEOPHYCEAE Cystoseira stricta	970.0±50	410.0±50	36.0±4	7.0±1	16.0±3	25.0±3	11.0±2	380.0±70
Pictyopteris membranacea	1100.0±300	430.0±20	89.0±7	18.0±2	39.0±7	85.0±8	65.0±6	720.0±150
RHODOPHYCEAE Spheerococus coronopifolius	700.0±50	330.0±30	27.0±4	7.1±1	12.0±4	55.0±8	55.0±7	26000.0±300
PHANEROGAMES Posidonia oceanica	600.0±30	270.0±20	57.0±7	7.0±1	16.0±4	130.0±15	100.0±10	690.0±120

Tableau : Radioéléments artificiels mesurés sur différents végétaux marins à la station de Villefranche le 01.06.1986.

Des calculs de périodes biologiques ont pu être établis sur Mytilus sp., espèce qui possède l'avantage d'être représentée à proximité de l'embouchure des fleuves et tout le long de l'année. C'est sur cette espèce que nous avons plus particulièrement porté notre attention ainsi que Posidonia océanica qui s'est également révélé être un bioindicateur de choix à l'échelle de la Méditerranée, ainsi que les phéophycées (Cystoseira sp. ET Dilophus membranacea) possédant des capacités d'accumulation plus importantes. (Figure 2).

Figure 2



IV. Objectives for the next reporting period:

- Poursuivre les campagnes de prélèvements sur le Rhône . Dépouiller l'ensemble des résultats obtenus en 1988 et réfléchir à l'établissement du bilan radioécologique du fleuve trois ans après Tchernobyl.
- Dépouillement et interprétation des résultats des expériences concernant le transfert du 60Co contenu dans des algues à des gammares et des larves de chironomes.
- Achèvement de la rédaction d'un mémoire de Thèse de Doctorat dont la soutenance est prévue pour la fin du premier trimestre de 1989. "Etude expérimentale de la fixation et de la rétention du 60Co par une algue planctonique dulçaquicole, Scenedesmus obliquus. Transfert à des organismes benthique et limicole".
- Travaux de synthèse sur l'ensemble des expériences relatives aux transferts du 60Co effectués au LREC dans le cadre du contrat avec la CCE.
- Rédaction d'un rapport de synthèse sur les résultats obtenus en Méditerranée.
- Effectuer une campagne de prélèvement sur le littoral Corse et une étude focalisée sur deux stations littorales rhodaniennes.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- CEN - SCK - Mol
- Université Catholique de Louvain
- Département de botanique de l'université de Liège.
- Département de modélisation mathématique de l'université de Liège.
- Discussion avec le LNETI au Portugal (Dr. BETTANCOURT).

VI. Publications:

FOULQUIER L., GRAUBY A., LAMBRECHTS A. & PALLY M. (188). Le concept de retour d'expérience en Radioécologie. Application au cas d'un fleuve à forte implantation nucléaire : le Rhône. In radiation protection practice. Seventh Internat. Congress of the Internat. Radiation Protection association. Sydney, 10-17 April 1988. Pergamon Press , Vol. 2, 633-636.

FOULQUIER L., LAMBRECHTS A. & PALLY M. (1988). Qualitative and quantitative evaluation of long-life radionuclides in the sediments, plants and fish of the Rhône river. Proceedings of a Seminar on the cycing of long-lived radionuclides in the biosphere : observation and models (Vol. 2). Madrid 15-19 September 1986. CCE and CIEMAT, 40 p.

LAMBRECHTS A., FOULQUIER L. & PALLY M. (1988). Etude comparée de l'impact radioécologique des installations nucléaires et de l'accident de Tchernobyl sur le fleuve Rhône. IVème Symposium INternational de Radioécologie, CEN Cadarache France, 14-18 Mars 1988 : 14 p.

NUCHO R., RAMBAUD A., FOULQUIER L. & BAUDIN J.P. (1988). Bioaccumulation du ^{60}Co par une algue planctonique Scenedesmus obliquus Türp. (Kûtz). Influence du stade de développement de la culture sur la fixation du radionucléide. Acta Oecologica : Oecol. Applic. 9 (2) : 111-125.

BAUDIN J.P. & FRITSCH A.F. (1988). Influence de la température sur l'accumulation par la voie directe du ^{60}Co chez un poisson dulçaquicole. Revue des Sciences de l'Eau, I (4) : 387-402.

BAUDIN J.P. & FRITSCH A.F. Relative contribution of food and water in the accumulation of ^{60}Co by a freschwater fish. Water Research (sous presse).

NUCHO R. & BAUDIN J.P. ^{60}Co retention by a planktonic alga, Scenedesmus obliquus. (en cours - Environmental Pollution).

Title of the project no.: 4

Behaviour of radionuclides in marine, freshwat environments. Radium transfert in freshwater ecosystems. Experimental studies and fields studies in the environment of french mining complex.

Head(s) of project:

GRAUBY, A et FOULQUIER, L

Scientific staff:

DESCAMPS, B ; BAUDIN-JAULENT, Y ; BRUNO, V ; REMILLET, J.N

I. Objectives of the project:

Ce projet vise à définir les risques radiologiques encourus par les populations de la Communauté Européenne du fait de l'extraction et du traitement du minerai d'uranium. Le programme est limité aux aspects liés à la radioécologie des eaux continentales. Le ^{226} Ra est le radionucléide étudié en priorité mais, pour les études de terrain, on s'intéresse aussi aux constituants de la famille du ^{238} U et en particulier au ^{210} Pb.

II. Objectives for the reporting period:

L'année 1988 a été consacrée au transfert du ^{226} Ra de l'eau vers une algue planctonique, Scenedesmus obliquus : cette algue représentant le maillon primaire, à la base de la chaîne alimentaire aboutissant aux poissons.

Deux aspects essentiels ont été étudiés :

- L'influence du niveau de contamination de l'eau sur la fixation par l'algue,
- L'influence du stade de développement de la culture au moment de la contamination.

Ces recherches ont été réalisées dans un laboratoire contrôlé, ventilé et en utilisant une boîte à gant à surface utile de 2 m^2.

III. Progress achieved:

A. Influence du niveau de contamination de l'eau sur le facteur de concentration du ^{226} Ra pour Scenedesmus.

Trois concentrations de l'eau ont été étudiées : 360, 3 600 et 36 000 $Bq.l^{-1}$. Les cinétiques de contamination des algues sont globalement identiques : montée très rapide pendant 1 à 3 jours puis décroissance et stabilisation à un niveau représentant entre 30 et 50 % du niveau initial de l'eau. Pour le facteur de concentration, exprimé en fonction du poids sec, les trois courbes ont des allures comparables (Figure 1). Le FC atteint une valeur de l'ordre de 75 000 après 1 à 2 jours, une diminution assez rapide survient ensuite elle est due à la dilution biologique, phénomène lié au mode de croissance par division des algues -. Une valeur constante de l'ordre de 10 000 est obtenue, pour les 3 expériences, après environ 20 jours de culture ; c'est cette valeur qu'il faut retenir pour l'échange eau - algue planctonique.

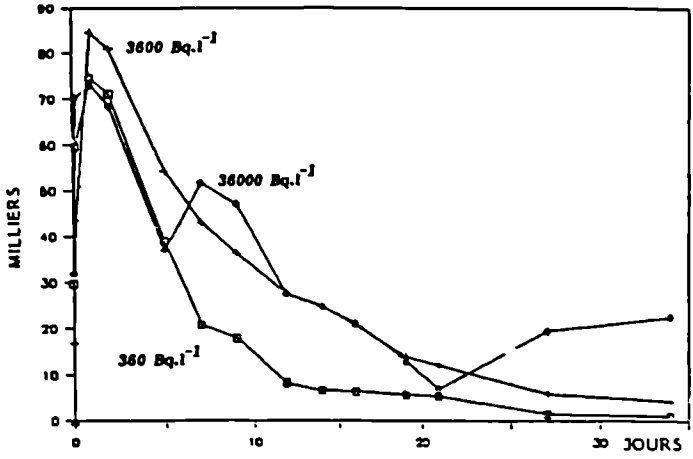


Figure 1 : Facteur de concentration de l'algue Scenedesmus obliquus: pour 3 niveaux de concentration de l'eau en ^{226} Ra. (Facteur de concentration exprimé en fonction du poids sec)

B. Influence du stade de développement de la culture au moment de sa contamination

Quatre stades de développement ont été étudiés :

- Contamination et début de culture sont simultanés (C0)
- Contamination après cinq jours de croissance (C5)
- Contamination après douze jours de croissance ((C12)
- Contamination après vingt-quatre jours de croissance (C24)

La figure 2 présente les quatre courbes obtenues pour le facteur de concentration. On constate l'existence de deux types de courbes : pour C0 et, à un degré moindre pour C5, on a un type d'évolution identique à celui présenté dans la figure 1 (obtention très rapide du maximum, lente diminution pour atteindre un état d'équilibre vers 10 jours) ; pour C12 et C24 l'état d'équilibre est atteint également après 10 jours, mais de façon lente et progressive. Dans les deux types de courbes le Facteur de concentration à l'équilibre est comparable : de l'ordre de 15 000.

Transposé sur le terrain ce résultat signifierait qu'une contamination aïgue de l'eau intervenant lors d'un bloom phytoplantonique aboutirait à une contamination rapide et importante de ces algues ; celles-ci seraient alors à la base de transferts trophiques divers pouvant aboutir aux poissons.

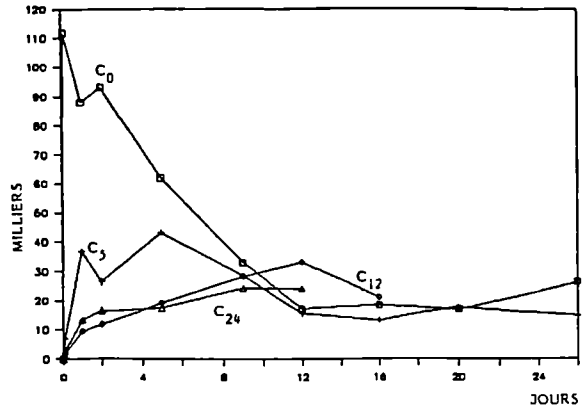


Figure 2 : Evolution du facteur de concentration pour 4 cultures contaminées à différents stades de leur développement

IV. Objectives for the next reporting period:

1/ Etudes expérimentales

Après l'échange eau - algue on réalisera les échanges eau - daphnie, algue - daphnie et daphnie - poisson.

2/ Etudes de terrain

Rapport de synthèse de l'étude du site du FOREZ (1986-1987). Cette étude comprenant trois aspects :

- prélèvements dans le lac et dans la rivière BESBRE,
- expérience "mousses aquatiques implantées",
- expérience "cages flottantes".

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Section de Radioécologie Physique (SRP) du SERE par l'intermédiaire de ses deux entités métrologie : le LMEI d'Orsay et l'antenne de Cadarache (M. PICAT, Chef de Section).

- Service de Protection et d'Instrumentation Nucléaire du Département de Protection Technique (DPT/SPIN) du C.E.N Fontenay-aux-Roses (M. ZETTWOOG, Chef de Service).

VI. Publications:

V. BRUNO, J-Y. GAL, B. DESCAMPS. Etude de la fixation du ^{226}Ra par une algue phytoplanctonique, Scenedesmus obliquus. Elément d'explication du rôle primordial de la chaîne alimentaire dans la contamination des poissons. Radioprotection (en cours de publication).

C. MICHEL. Le radium 226. Etude bibliographique sur l'incidence radioécologique aquatique. Etude expérimentale sur la contamination des carpes par une eau de lixiviation chargée en radium. Thèse Vétérinaire. Toulouse (Juillet 1988).

Title of project no.: 5

Cycling of tritium:
Transfert aux plantes de l'hydrogène tritié, du méthane tritié et des molécules tritiées les plus importantes qui peuvent en dériver. Etude de laboratoire et de terrain. Modélisation

Head(s) of project

Y. BELOT

Scientific staff:

C. CAPUT
J. GUENOT

I. Objectives of the project:

Le but du travail est d'étudier la dynamique de captation et de rétention par les plantes de l'hydrogène tritié, du méthane tritié et des molécules tritiées les plus importantes qui peuvent en dériver, en relation avec les paramètres climatiques et physiologiques et la composition de l'atmosphère. Le travail comporte des expériences de laboratore et de terrain ainsi que des tentatives de modélisation.

II. Objectives for the reporting period:

Au cours de l'année 1988, nous avons continué l'étude de la captation du formaldéhyde par la partie aérienne des plantes. Des études biochimiques ont été effectuées pour identifier les composés organiques sur lesquels se fixe le formaldéhyde. Par ailleurs une méthode a été mise au point pour déterminer la concentration du formaldéhyde en présence d'eau tritiée dans divers types d'effluents.

III. Progress achieved

I) Etude en chambre expérimentale de la captation du formaldéhyde par la partie aérienne des végétaux

Les travaux effectués en 1986 et 1987 nous ont montré que la vitesse de dépôt de l'hydrogène et du méthane tritiés sur les feuilles des végétaux était très faible et généralement inférieure à 10^{-6} cm/s. Ces molécules concourent très peu ou peut-être pas du tout à l'incorporation du tritium dans la matière organique des végétaux. Nous avons alors été amenés à examiner si certains dérivés carbonylés tels que le formaldéhyde pouvait servir de véhicule au tritium ou au carbone-14 et permettre leur incorporation dans la matière sèche des plantes. Des essais préliminaires réalisés en 1987 nous ont montré que la vitesse de dépôt de ce dérivé sur des feuilles de plante était effectivement très élevée et pouvait atteindre 0,2 cm/s.

Au cours de l'année 1988, nous avons cherché à connaître les mécanismes par lesquels le formaldéhyde se fixait sur la matière organique des végétaux. Ce travail a été réalisé en collaboration avec le laboratoire de chimie biologique de l'Institut National de la Recherche Agronomique situé à Grignon. Les expériences réalisées ont consisté à marquer des plants de tournesol par du ^{14}C-formaldéhyde puis a déterminer par des méthodes classiques de biochimie les composés dans lesquels le ^{14}C était incorporé. Le marquage des plantes a été réalisé en exposant leur partie aérienne à un flux d'air contenant 10^5 Bq/m^3 de formaldéhyde radioactif et 2 µg/m^3 d'entraîneur stable pendant une durée de 3 heures. Après un laps de temps variable les feuilles exposées ont ensuite été coupées, fixées dans l'azote liquide et lyophilisées avant d'être analysées. L'extraction des petites molécules marquées a été réalisée en utilisant utilisant de l'éthanol à 50 % selon le protocole de Schürman (1969). Cet extrait a été soumis à une séparation bidimensionnelle qui consiste en une électrophorèse sur couche mince suivie d'une double chromatographie.La détection des produits marqués a été faite par autoradiographie. Les composés marqués ont été récupérés par grattage de la couche mince puis tansférés dans des fioles de comptage pour détermination quantitative de leur radioactivité.

La répartition de la radioactivité entre l'extrait alcoolique et le résidu d'extraction varie suivant les échantillons. La fraction extractible constituée de petites molécules est en moyenne de 44 %, alors que la fraction résiduelle constituée majoritairement de protéines membranaires est de 56 %. Les cartes métaboliques obtenues sur les extraits alcooliques par séparation bidimensionnelle révèlent 4 groupes de taches radioactives.Un premier groupe de faible importance (1-2 %) correspond aux substances qui n'ont pas migré. Un deuxième groupe (2-10 %) correspond à une substance qui migre exclusivement par chromatographie et qui a été identifiée comme du formaldéhyde libre. Un troisième goupe (6-8 %) est vraisemblement constitué par du glycolate et du glycérate marqués. Le qua-

trième groupe le plus important (80-90 %) a été identifié comme étant formés par des produits d'addition du formaldéhyde et des acides aminés c'est-à-dire les produits de la forme CH_2= N - R - COOH. Nous avons montré que ces composés d'addition pouvaient être révélés chimiquement par le réactif habituel des acides aminés, que leur comportement en chromatographie était le même que celui de acides aminés, mais que leur comportement en électrophorèse était différent (moindre migration).

Le formaldéhyde capté par les plantes après une exposition de quelques heures et un temps d'attente de 24 heures au maximum, se trouve fixé sur des composés solubles (principalement des acides aminés) ou sur des composés insolubles (probablement des protéines membranaires). La présence d'une petite proportion d'acides organiques marqués, glycolate et glycérate, semble indiquer un début de métabolisation. Pour vérifier cette hypothèse il conviendrait de réaliser des expériences plus longues.

2) Détermination du formaldéhyde tritié dans l'atmosphère ou les effluents des installations nucléaires

Une méthode d'analyse a été mise au point pour séparer le formaldéhyde tritié de l'eau tritiée dans des échantillons d'origine diverse. Dans le cas d'effluents gazeux ou de l'atmosphère, on concentre l'eau tritiée et le formaldéhyde dans 200 ml de solution à pH 5 contenant 17 mg de formaldéhyde stable ajouté comme entraîneur. On compte la radioactivité totale de la solution contaminée puis on précipite un dérivé du formaldéhyde en ajoutant 165 mg de dimedon (5,5-dimethylcyclohexanedione-1,3) dissous dans 2 ml d'éthanol. Après avoir laissé le précipité reposer pendant une nuit, il est recueilli sur un filtre puis séché jusqu'à poids constant. Le dérivé du dimedon est ensuite brûlé dans un four pour obtenir de l'eau dont on mesure la radioactivité.

Cette méthode a été appliquée pour déterminer les impuretés radioactives contenues dans des bouteilles de méthane tritié ou d'hydrogène tritié fournies par le C.E.N. de Saclay. L'activité totale était de 1,1 10^9 Bq/m^3 dans le premier cas et de 1,3 10^8 Bq/m^3 dans le second cas. Dans les deux cas nous avons trouvé sensiblement la même proportion d'impuretés, soit 8 10^{-3} d'eau tritiée et 2 10^{-5} de formaldéhyde tritié.

IV. Objectives of the next reporting period

L'objectif des travaux réalisés en 1989 sera d'identifier et de déterminer la concentration du formaldéhyde tritié dans des effluents liquides ou gazeux de différentes origines en utilisant la méthode d'analyse mise au point cette année. Une publication sera par ailleurs préparée sur les résultats obtenu en 1988.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]

Laboratoire de Chimie Biologique de l'Institut National de la Recherche Agronomique
78850 Thiverval-Grignon (Professeur C. Costes; chercheur Mme Jolivet)

VI. Publications

Title of the project no.: **6**

Impact des radionucléides relâchés en conditions accidentelles.

Head(s) of project:

M. HUGON

Scientific staff:

M. HUGON
M. MAUBERT
S. ROUSSEL

I. Objectives of the project:

Devenir dans l'environnement des produits de fission rejetés en cas d'accident.

II. Objectives for the reporting period:

cf. Rapport d'activité de 1987

2.1 - **Travaux de laboratoire**

Poursuite de l'expérience de production d'aérosols à haute température. Amélioration du four disponible.

2.2 - **Travaux de terrain**

Poursuite de l'étude des dépôts consécutifs à l'accident de Tchernobyl.

III. Progress achieved:

Point 1 : Travaux de laboratoire

Le four décrit dans le précédent compte-rendu d'activité a été mis en oeuvre. Il s'agissait de porter un mélange représentatif d'une pastille de combustible nucléaire à une température de 2 000°C pour générer des aérosols représentatifs du terme source accidentel.

Point 2 : Travaux de terrain

Le travail sur les données recueillies dans le bassin du Var en 1986 et 1987 s'est poursuivi.

Un ajustement mathématique plaqué sur les valeurs mesurées a permis de modéliser l'évolution de la radioactivité dans 8 éléments de la ration alimentaire après le dépôt pour 5 radioéléments : ^{131}I, ^{134}Cs, ^{137}Cs, ^{103}Ru et ^{106}Ru. Cette évolution est représentée pour le ^{137}Cs figure 3.

En multipliant ces valeurs par les quantités ingérées on a pu calculer la quantité de radioactivité incorporée quotidiennement par un individu qui consommerait exclusivement des produits locaux, et de là évaluer l'équivalent de dose engagé.

Pour une période de 1 an après le dépôt l'exposition ainsi calculée est de 200 μ Sv. La ventilation par radionucléide et par élément de la ration alimentaire est représentée figure 4.

Une publication au Symposium de Mars à Cadarache reprend de façon synthétique les principaux résultats obtenus lors de l'étude du bassin du Var.

IV. Objectives for the next reporting period:

4.1 - Poursuite de l'expérience sur la production d'aérosols et étude de leur incorporation par les plantes.

Le dispositif de production d'aérosols sera utilisé pour contaminer des plantes, jeunes arbres et produits de l'agriculture. On étudiera le pouvoir de pénétration des radionucléides dans les plantes, ainsi que leur période effective de résidence.

Des contaminations seront effectuées sur des végétaux à divers stades de développement afin de mesurer l'influence de la période à laquelle se produit le dépôt sur la concentration dans les récoltes à mâturité.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Laboratoire de Chimie inorganique et Nucléaire

Prof. C. RONNEAU
Université Catholique de Louvain
Chemin du Cyclotron 2
B-1348 LOUVAIN LA NEUVE

VI. Publications:

C. RONNEAU ; E. FAGNIART ; K. FONSNY ; P. ANDRE ;
C. MYTTENAERE ; A. DEBAUCHE ; J.M. LAMBOTTE ; H. MAUBERT ;
Contamination des écosystèmes forestiers par le césium.
IVème Symposium International de Radioécologie de CADARACHE.
Mars 1988.

H. MAUBERT ; S. ROUSSEL ; R. LION ;
Les dépôts radioactifs consécutifs à l'accident de Tchernobyl dans le bassin du VAR.
IVème Symposium International de Radioécologie de CADARACHE.
Mars 1988.

Title of the project no.: 7

Etude comparative du transfert sol-plante du neptunium, du plutonium et de l'américium. Comparaison de la distribution et du transfert des radionucléides dans l'environnement terrestre en France et en Grande-Bretagne.

Head(s) of project:

A. GRAUBY

Scientific staff:

J. DELMAS, C. COLLE

I. Objectives of the project:

Ce programme a deux objectifs principaux :

1/ La détermination, dans les conditions du milieu naturel, des dépôts sur le sol et la végétation des radionucléides (Pu, Am, Cs) issus des retombées atmosphériques des essais d'armes nucléaires.

2/ L'étude expérimentale du transfert sol-plante du neptunium et de l'américium en fonction des caractéristiques chimiques des sols et des types de végétaux.

II. Objectives for the reporting period:

1/ Prélèvements périodiques d'échantillons de végétaux (luzerne, Ray-grass) et d'eau de pluie sur les huit parcelles implantées dans le milieu naturel dans quatre régions du territoire français.

2/ Réalisation des analyses des différents radioéléments pour les échantillons prélevés à la fin du deuxième semestre 1987 et au cours du premier semestre 1988 (sols, végétaux, eau).

3/ Poursuite de l'étude expérimentale des transferts sol-plante du neptunium et de l'américium sur trois types de sols.

4/ Intercomparaison des résultats obtenus par le C E A Cadarache et le Laboratoire d'Harwell avec qui ce programme est mené en collaboration.

III. Progress achieved:

Au cours de l'année 1988 la collecte périodique des échantillons de végétaux et d'eau de pluie s'est poursuivie sur l'ensemble des parcelles de plein champ.

Parallèlement, les analyses des différents radioéléments (Cs, Pu, Am) ont été réalisées sur les échantillons de sol prélevés à la fin de l'année 1987 et sur les échantillons d'eau et de végétaux récoltés durant le deuxième semestre 1987 et le premier semestre 1988. Les résultats obtenus indiquent des concentrations en césium 137, en plutonium 239 + 240, en plutonium 238 et en américium 241 inférieures respectivement à 50, 0.70, 0.040 et 0.27 Bq par Kg de sol sec pour la zone de 0 à 30 cm de profondeur. En ce qui concerne les mesures effectuées sur les eaux de pluie et les végétaux il n'apparaît pas de différences significatives entre les échantillons récoltés à proximité des installations nucléaires et ceux issus des zones éloignées.

Pour ce qui est de l'étude expérimentale des transferts sol-plante du neptunium et de l'américium sur trois types de sols, les valeurs des facteurs de transfert (calculés par rapport à la matière séche) sont portées dans le tableau suivant :

		Sol acide		Sol calcaire		Sol organique	
		^{241}Am	^{237}Np	^{241}Am	^{237}Np	^{241}Am	^{237}Np
Salade : feuilles		$1,8.10^{-2}$	1.10^{-1}	$5,7.10^{-3}$	$5,1.10^{-3}$	$2,6.10^{-3}$	$2,8.10^{-2}$
Radis	Feuilles	$9,1.10^{-2}$	$4,2.10^{-1}$	1.10^{-2}	$3,8.10^{-2}$	$1,7.10^{-2}$	$9,4.10^{-3}$
	Racines	7.10^{-3}	$5,7.10^{-2}$	$8,7.10^{-4}$	$2,4.10^{-2}$	$1,1.10^{-3}$	$1,4.10^{-2}$
Haricot	Feuilles + Tiges	$4,4.10^{-3}$	$2,2.10^{-2}$	$7,3.10^{-4}$	$6,3.10^{-3}$	$1,4.10^{-3}$	$8,1.10^{-3}$
	Fruit	$6,1.10^{-4}$	5.10^{-3}	$1,2.10^{-4}$	$9,7.10^{-4}$	7.10^{-5}	$1,2.10^{-3}$

Progress achieved : (suite)

Ces résultats indiquent une influence marquée des propriétés du sol acide sur l'absorption racinaire des deux nucléides. En effet, les transferts sont souvent supérieurs d'un ordre de grandeur par rapport à ceux résultant des deux autres types de sol.

A la fin de 1988 des prélèvements ont eu lieu en vue d'effectuer la séparation des différentes formes physico-chimiques du neptunium et de l'américium contenus dans ces sols.

IV. Objectives for the next reporting period:

1/ Poursuite des récoltes de végétaux et des collectes d'eau de pluie sur les parcelles implantées dans le milieu naturel. Analyses des teneurs en Cs, Pu et Am sur ces échantillons.

2/ Détermination du transfert sol-plante de l'américium et du neptunium pour une plante fourragère pluriannuelle (luzerne) sur trois types de sol. Recherche des formes physico-chimiques de ces deux nucléides dans ces sols.

3/ Echange des résultats et rencontres périodiques entre le Laboratoire de Cadarache et celui d'Harwell.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Centre de recherches d'Harwell (Environmental and Medical Sciences Division).

Notre collaboration s'effectue avec Monsieur P.A. CAWSE

VI. Publications:

P.A. CAWSE, C. COLLE, Comparison of radionuclide deposition to soil and vegetation. Actes du IVè Symposium International de Radioécologie de Cadarache, 14-18 mars 1988, tome 1, D75 - D89.

Title of the project no.: 8

Dépôt des radionucléides sur la végétation et les sols

Head(s) of project:

A. GRAUBY

Scientific staff:

J. DELMAS - C. COLLE

I. Objectives of the project:

Ce programme a trois objectifs :

1/ Comparaison des transferts aux végétaux du césium et du cobalt dans le cas d'une contamination chronique et d'une contamination accidentelle ;

2/ Recherche des possibilités de réduire les transferts résultant d'une contamination accidentelle en utilisant des techniques culturales particulières ;

3/ Comparaison des situations pour deux conditions climatiques différentes : climat continental humide (Jülich, RFA) et climat méditerranéen sec (Cadarache, FRANCE).

II. Objectives for the reporting period:

Depuis la mise en place de ce programme trois thèmes de recherche ont été développés :

1/ Evaluation des facteurs de transfert sol-plante du césium 137 et du cobalt 60 résultant d'une contaminaiton homogène de la zone de labour (0-30 cm). Détermination de l'influence de la concentration du sol en césium et cobalt stables sur l'absorption racinaire par les végétaux des radionucléides correspondants.

2/ Etude du transfert aux végétaux du césium 134 et du cobalt 57 à la suite d'un dépôt important de ces radioisotopes sur la surface du sol ou sur les parties aériennes des plantes.

3/ Détermination de l'influence de trois types de labour (superficiel, normal et profond) sur les transferts sol-plante du césium 134 et du cobalt 57 à partir d'un sol ayant reçu une contamination accidentelle.

III. Progress achieved:

Les différentes expérimentations réalisées depuis le début de ce programme ont concerné les quatre thèmes suivants :

1/ Détermination des facteurs de transfert sol-plante du césium et du cobalt pour des cultures de céréales (blé, orge), de carotte et de salade réalisées en lysimètres (0,5 x 0,5 x 0,6 m) à partir d'un sol brun calcaire et où la contamination est répartie de façon homogène dans les 30 premiers centimètres. L'apport des radionucléides correspond à un dépôt au sol de 3.10^7 Bq par m^2.

Les facteurs de transfert sol-plante (calculés par rapport à la matière sèche) ont été les suivants :

Cobalt 60 :	Blé (paille) = $9{,}6.10^{-3}$	Blé (grains) = $7{,}2.10^{-3}$
	Orge (paille) = $9{,}4.10^{-3}$	Orge (grains) = $7{,}3.10^{-3}$
	Carotte (feuilles) = $4{,}6.10^{-2}$	Carotte (racines) = $4{,}7.10^{-2}$
	Salade (feuilles) = $6{,}5.10^{-2}$	
Césium 137 :	Blé (paille) = $7{,}3.10^{-3}$	Blé (grains) = $4{,}2.10^{-3}$
	Orge (paille) = $7{,}1.10^{-3}$	Orge (grains) = 4.10^{-3}
	Carotte (feuilles) = $7{,}6.10^{-2}$	Carotte (racines) = $6{,}4.10^{-2}$
	Salade (feuilles) = $5{,}4.10^{-2}$	

2/ Influence d'un apport dans le sol de cobalt et de césium stables (50 et 10 ppm respectivement) sur le transfert sol-plante des radioisotopes correspondants (cobalt 60 et césium 137).

Il est apparu que la présence des éléments stables provoque une augmentation de l'absorption racinaire d'un ordre de grandeur environ. Les facteurs de transfert sol-plante qui ont résulté de cette expérimentation sont les suivants :

Progress achieved : suite 1/

Cobalt 60 :	Blé (paille) = $7,3.10^{-2}$	Blé (grains) = 3.10^{-2}
	Orge (paille) = $6,9.10^{-2}$	Orge (grains) = $2,9.10^{-2}$
	Carotte (feuilles) = $9,7.10^{-2}$	Carotte racines) = $2,1.10^{-1}$
	Salade (feuilles) = $1,2.10^{-1}$	
Césium 137 :	Blé (paille) = $8,8.10^{-2}$	Blé (grains) = $6,1.10^{-2}$
	Orge (paille) = 9.10^{-2}	Orge (grains) = $6,4.10^{-2}$
	Carotte (feuilles) = $5,8.10^{-1}$	Carotte (racines) = $5,3.10^{-1}$
	Salade (feuilles) = $3,1.10^{-1}$	

3/ Comparaison des transferts racinaires résultant d'une contamination chronique du sol avec les transferts provoqués par une contamination accidentelle touchant soit le sol soit les organes aériens des plantes.

Ces travaux ont été réalisés sur des cultures de blé et de carotte. La contamination chronique correspond à 3.10^7 Bq par m^2 de césium et de cobalt mélangés dans les 30 premiers centimètres du sol (soit 75.10^3 Bq par Kg de sol sec). La contamination accidentelle résultait d'un apport par l'eau d'irrigation sur les végétaux ou le sol de la même quantité de radioisotopes (3.10^7 Bq par m^2).

Les transferts observés ont été les suivants :

		Contamination	Contamination accidentelle	
			sur le sol	sur la végétation
Cobalt	Blé (grain) Bq/g sec	0,8	1,8	325
	Carotte (racine) Bq/g sec	3,6	97,5	3 542
Césium	Blé (grain) Bq/g sec	1,2	8	500
	Carotte (racine) Bq/g sec	5,7	105,6	5 336

Progress achieved : suite 2/

La comparaison de ces différents résultats montre qu'un dépôt accidentel de césium ou de cobalt ayant lieu soit sur le sol soit sur la végétation provoque des transferts plus importants que ceux issus d'une contamination de même intensité mélangée de façon homogène dans les 30 premiers centimètres du sol.

4/ Recherche de l'influence de 3 types de labour sur l'absorption racinaire du cobalt et du césium.

A la fin de l'année 1987, les sols qui avaient reçu en surface un apport accidentel de césium et de cobalt ont été partagés en trois lots sur lesquels trois modes de travail du sol ont été testés :

- Un travail superficiel touchant les dix premiers centimètres,

- Un labour normal afin d'enfouir à 30 centimètres de profondeur la zone contaminée,

- Un labour profond à 50 centimètres de profondeur.

Une culture de blé de printemps a été mise en place en 1988 sur les sols ainsi travaillés. Les activités de cobalt et de césium mesurées sur les grains de blé à maturité ont été les suivantes :

Travail superficiel du sol : cobalt = $2,58 \pm 0,6$ Bq par gramme
césium = $3,78 \pm 0,8$ Bq par gramme

Labour normal (30 cm) : cobalt = $0,49 \pm 0,4$ Bq par gramme
césium = $0,65 \pm 0,6$ Bq par gramme

Labour profond (50 cm) : cobalt = $0,09 \pm 0,01$ Bq par gramme
césium = $0,04 \pm 0,01$ Bq par gramme

Ces valeurs montrent que, dans le cas d'une plante qui possède un enracinement en grande partie superficiel, le labour se révèle être efficace pour diminuer le transfert au grain à partir d'un sol qui a reçu un dépôt important de césium ou de cobalt en surface. Dans le cas de notre expérimentation ce phénomène a certainement été amplifié par le fait que les cultures de blé ont reçu, tout ou long de la végétation, des irrigations fréquentes mais de faibles intensités qui ont favorisé le développement racinaire dans la zone superficielle du sol.

IV. Objectives for the next reporting period:

Poursuite des essais pour déterminer l'efficacité des différents types de labour sur la diminuation des transferts racinaires résultant d'un dépôt accidentel de nucléides à la surface d'un sol. Depuis le deuxième semestre 1988 des cultures de luzerne ont été mises en place sur les sols labourés qui avaient reçu précédemment du blé de printemps.

Un suivi du transfert racinaire du césium et du cobalt sera effectué en fonction des récoltes.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Institut de Recherches en Agronomie de Jülich (RFA)

Chef : F. FUHR

Cette collaboration s'effectue avec Messieurs W. STEFFENS et W. MITTELSTAEDT.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Natural Environment
Research Council
Polaris House
North Star Avenue
GB- Swindon SN2 1EU Wilts

Contract no.: BI6-B-038-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr E.I. Hamilton
Inst. Marine Env. Research
Prospect Place - The Hoe
GB- Plymouth Devon PL1 3DH

Telephone number: 0752/221.371

Title of the research contract:

The role of surfaces in the transport of radionuclides in the marine environment.

List of projects:

1. The role of surfaces in the transport of radionuclides in the marine environment.

Title of the project no.:

The role of surfaces in the transport of radionuclides in the marine environment.

Head(s) of project:

Dr. E.I. Hamilton
Plymouth Marine Laboratory
Prospect Place, West Hoe
Plymouth, Devon PL1 3DH, U.K.

Scientific staff:

i Mr. R.J. Clifton

I. Objectives of the project:

Investigation and definition of the nature of important surfaces (physical chemical and biological) which control the distribution of radionuclides in the marine environment. It is assumed that some surfaces are more suitable for the uptake of radionuclides than others; an objective is to identify the nature of such surfaces in relation to changes in redox state, pH and biological productivity in the retention, loss and recycling of radionuclides in estuaries, the near shore environment and the oceans.

II. Objectives for the reporting period:

Using validated novel methods to provide data in order to illustrate whether or not a single "surface" can account for the retention of radionuclides and to identify that surface. Systematic studies will be carried out initially on magnetite, calcite, "colloids" and ferromagnese minerals in relation to uptake or radionuclides paying attention to pH, ageing and electrophoretic mobility. To continue work in the Atlantic Ocean by examining surfaces throughout the water column at one station at least in order to relate uptake of radionuclides with biological activity and processes. To complete limited studies on the distribution of radionuclides in SW England in relation to the uptake on surfaces ie Chernobyl debris.

III. Progress achieved: Methodology-1) Esk estuary, Cumbria studies. Surface sediments are screened for alpha emitters using CR39 autoradiography (detection limit 1-2x10-5 alphas/cm2/sec), plus estimates of emanation rates. Individual particles of sediment are removed, chemically extracted, then subject to surface barrier analysis for Po,Ra,U,Th,Pu,Am and Cm radionuclides. Element analysis by X-ray fluorescence and single grain analysis for Fe,Mn by microcolorimetry. Non-evasive techniques for separating sediment grains have been developed. 2) NE Atlantic studies. CR-39 alpha autoradiography and element analysis by XRF. 3) Sequential leaching of sediments using conventional methods. 4) Surface area of sediments is determined by the BET method, and protonation by tritiated water. 5) A 242-Cm source is used in a Rutherford backscatter system for surface element profiling of sediments.

Results and Discussion. In the surface sediments of the Esk a very significant proportion of alpha-radionuclides in BNF plc low level waste are associated with one phase; for the present morphological classification they are termed "organoliths". They have mean particle diameters of 0.2-2mm and are coated with a layer of Fe-Mn hydrated oxides. Bulk composition is: 82%SiO_2, 12%Fe_2O_3, 4%MnO, 40ppm S, 3%C and 0.2%N. Alpha radioactive radionuclides associated with the organoliths exhibit a diffuse distribution, hence they are derived from conservative species. Conventional methods of treating sediments usually destroy organoliths, hence then have not been observed before. It is probable that much of the alpha radioactivity in coarse sediments can be accounted for by the presence of detrital Fe-Mn organolith debris. Organoliths are a feature of the sediment-water interface; Fe and Mn are derived from underlying anaerobic sediments via "pore liquid" transfer. Surface alpha particle activity is x10-100 greater than for quartz which constitutes c90% of sediment. Single particle analysis for alpha emitters has been accomplished for organic debris, coal, coke, magnetite, haematite, ilmenite, also for igneous, biogenic and Permo-Trias desert quartz, aluminosilicates and foraminifera which are the most common constituents of the sediment. A mass balance for alpha emitters is in progress, which allows for alpha emitters in thick sources (eg organoliths) which are not detected by CR-39 techniques. Preliminary data for organoliths indicate the formation of FeMn crusts, as found in ferromanganese nodules (present in the NE Irish Sea). The single particle approach is being investigated in relation to alpha recoil processes, eg 238Pu&239+240Pu separations. By the end of this contract research on surfical sediments of the Esk, together with preserved historical sediments deposited over the past 50 yrs should be completed, together with a preliminary evaluation of the presence or absence of organoliths in adjacent areas of the NE Irish Sea. For the present, concepts based on Kd and sequential leaching are discarded. The Kd concept is valuable in large area modelling as the details of the sediment processes constitute constants in box-transfer processes. However, in relation to climatic and geomorphological change there is some doubt concerning the reliability of some modelling procedures when applied over the next 10-10,000 years, an area of concern in radiological protection. If the processes related to organolith production ceases, then it is possible that levels of radioactivity in the Esk could be reduced by a factor of 10-100. Data for various types of single particle analysis are given in Table 1. Uranium particles are also being observed; those with emanation relate to natural minerals, with no emanation but high Pu,Am to BNF hot particles, with high U but low emanation probably to BNF releases of depleted U. A few high emanation low Pu,Am, U types may represent 226Ra-210Po debris from the local manufacturer of fertilisers.

Sequential leaching procedures. Using fresh Esk sediment a 0.5x10cm column of sediment was sedimented to reproduce deposition for half a tidal cycle, then sectioned (1cm) and radionuclides were determined in the aliquots. The specific radioactivity of all radionuclides decreased top to bottom of the core; 144-Ce was concentrated in the top 1 cm; the 137Cs/241Am ratio decreased linearly (top to bottom) and was highly correlated with organic content (r=0.97); other observed correlations were: 0.98-to surface area; 0.92-exchange protons, indicating the importance of surface exchangable sites. Various artifacts in the tritium exchange technique are being considered further. Sequential leaching

procedures indicate that different radionuclides are associated with different components of the sediment, but as no data are provided by the technique concerning the composition of the sediment the radionuclide partitioning data has a limited usefulness.

Alpha Rutherford Backscatter. Due to the decay of the Cm-242, 6-8 days counting time are now required. The depth of element profile analysis is about 6 m for organics and 2 m for inorganic constituents. Examination of dry Esk sediment indicates an element profile containing C and N, but not for quartz which constitutes 80% of the sample, hence it is possibly covered by an organic coating. The presence of sharp alpha energies relate to Rn&Th emanation from the sediment, a feature which is being considered further as their presence interferes with the Fe and Mn signals. Atlantic Ocean- Porcupine Sea Bight 51.04N12,39.4W Sept 1988; sediment core, 2000m depth. Using long exposures to CR39, corrected for fading of latent image, alpha particle distributions down the core relate to mobility of Rn and Th in the top 4cm (oxic portion). Fractional deposition of foraminifera in the top 48cms results in a infaunal process which probably disturbs radionuclide profiles. Data are presented in Table 2 illustrating some of the results obtained sofar. For the April 1988 cruise a large number of samples of biota, faeces etc. have been prepared for CR-39 exposure to determine partitioning of alphas between water and particulates; data will be available late 1990. Studies continue on Chernobyl radioactivity in SW England; some special surfaces for uptake of 137Cs have been identified. Recent sediment of the Esk contain c6Bq.kg 237-Np reflecting conservative nature of this radionuclide.

Table 1. Examples of some radionuclide data for single particles of Esk sediment, 1987. (Bq.1g dry wt and ppm for U.)

Material	239+240Pu	238-Pu	9/8Pu	241Am	Pu/Am *	210Po	U(ppm)
Total sediment	1.34	0.30	4.5	1.72	0.8	0.007-0.02	3-6
organolith n/4	5.50	3.38	1.6	2.04	2.7	0.1	-
n/22	7.20	1.34	5.3	8.64	0.8	0.1	-
n/33	2.7	0.50	5.4	1.08	2.5	-	-
n/41	0.42	0.22	1.9	1.82	0.2	0.01	2
A/S	1.18	0.30	3.9	2.32	0.5	0.01	-
B/S	2.22	0.26	8.5	3.14	0.7	-	415
Igneous quartz	c0.03	<0.02	-	-	-	-	< 0.01
Organic debris	0.56	0.14	4.0	0.60	0.9	-	c 0.01
Coke debris	0.28	0.14	2.0	0.38	0.8	-	2.1
Coal debris	0.08	<0.03	-	2.24	-	-	18.7

*239+240Pu/241Am

Table 2. Data for Atlantic sediment core- 12.5cms x 4cms

Depth cm	0	1	2	3	4	5	6	7	8	9
Redox	◄			oxic			►	◄	anoxic	►
Density	◄		► 0.6g cm3	◄	►	◄	c6.5g cm3	◄		►
Element	◄► Mn loss	◄	►Increase Mn, 226Ra ►230Th variable. ◄Mn,Ra,Th							►
	◄ excess 234Th ► (No excess 234Th no Be-7 detected)									►
	◄ 210-Pb		►							
Total alpha activity	◄► decrease	►	◄ increase	►	◄	decrease				►
Forams. gms 1cm section	◄► 0.37	◄	0.53-0.65				►	◄	0.8-1.1	►

IV. Objectives for the next reporting period:

Characteristics (physical, chemical, radiometric, biological) of organoliths will be defined. The age of the major types of organolith will be determined by radiometric methods in order to evaluate their residence times in different environments. Microchemical (organic and inorganic) and electrochemical techniques will be used, together with magnetic studies and the possible involvement of bacteria. Using data, observations and experience already acquired the study will be extended to other areas of the NE Irish Sea. Organoliths seem to be unique to the Esk;their origin will be studied in terms of any association with BNF wastes and natural sources, eg Permo-Triassic sediments. Kd concepts will be re-evaluated in terms of fractal properties.

Atlantic Ocean samples, being exposed to CR-39, will be developed and examined when appropriate. Research continues on Chernobyl debris in SE England.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

It is hoped that collaboration will continue with MAFF fisheries Directorate, Lowestoft, UK, and other research organisations as required by the research.

VI. Publications:

Hamilton, E.I. (1987) The origin and distribution of 'hot particles' derived from the nuclear industry and dispersed in the environemnt. 1987 DoE Contract PECD7/9/265 Sector No: 5.1 pp 109. (For practical and logistical reasons part of the CEC research was developed in relation to the DoE contract).

Hamilton, E.I. (1988) Geobiocoenosis: The chemical elements and relative abundances in biotic and abiotic systems. Sci. Total Environment 7 253-267.

Hamilton, E.I. (1988) Migration of nuclides in the environment. Plenary lecture - Royal Soc. Chem. Int. Conf. on Nuclear and Radiochemistry, 11-15th July 1988, Brighton UK. (A paper on organoliths and the Esk which will be published in Radchim. Acta.)

Hamilton, E.I. (1989) Terrestrial radiation - an overview. (Accepted for publication Rad. Phys and Chem).

Clifton, R.J., Stevens, H.E. and Hamilton, E.I. (1989) Uptake and depuration of 241-Am, 239+240Pu, 238-Pu, 137-Cs and 106-Ru by Mytilus edulis under natural stress. (Accepted for publication in Marine Ecology Progress Series.)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Nat. Environment Research Council
Polaris House, North Star Avenue
GB- SWINDON, Wiltshire, SN2 1EU

Contract no.: BI6-B-233-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. O.W. Heal
Merlewood Research Station
Institute of Terrestrial Ecology
Grange-over-Sands
GB- Cumbria LA11 6JU

Telephone number: (04484) 2264

Title of the research contract:

The Relationship between Soil Organic Matter and the Actinide Elements

List of projects:

1. The Relationship between Soil Organic Matter and the Actinide Elements.

Title of the project no.:

The relationship between soil organic matter and the actinide elements. Contract No BI6-B-233-UK

Head(s) of project:

F R Livens

Scientific staff:

D L Singleton

I. Objectives of the project:

Earlier studies (Livens et al, 1987; Livens & Baxter 1988) have identified significant association of artificial actinides with soil organic matter. In particular:

1) isolation on a large scale of actinide-containing humic and fulvic acid fractions;
2) characterisation of these fractions principally by gel permeation but also by conventional chemical methods, ultrafiltration, ion exchange and other appropriate techniques.

II. Objectives for the reporting period:

1) Isolation of several grams each of humic and fulvic acids containing substantially elevated levels of artificial actinide elements;
2) Optimisation of the gel permeation technique to provide the best possible resolution of humic and fulvic acids and permit determination of the nominal molecular weight of each fraction;
3) Physical isolation of individual fractions of the humic and fulvic acids;
4) Determination of the actinide distribution in these fractions.

III. Progress achieved:

a) Progress to date

Humic and fulvic acids have been isolated on a large scale from an alluvial gley soil which previous studies (Livens & Baxter, 1988) have shown to contain considerably elevated levels (5-10 k Bq kg^{-1}) of plutonium and americium as a result of discharges of liquid waste from the Sellafield reprocessing plant. The field-moist soil was wet-sieved through a 2 mm mesh, then extracted with 0.5 M NaOH. The extract was centrifuged and acidified to precipitate humic acid. The humic acid was centrifuged off, washed and freeze-dried. The supernatant, containing the fulvic acid fraction, was dialysed against distilled water to remove salts, then freeze-dried. During dialysis, a part of the fulvic acid fraction passed through the dialysis membrane. This is referred to as 'dialysable fulvic acid'. In total, 15% by weight of the sample, containing 23% of the total plutonium, is leached by the alkali extraction. The distribution of organic matter and plutonium between the various fractions is shown in Table 1.

Table 1. Distribution of organic matter and Pu between fractions

	Organic Matter (%)	Pu-239, 240(%)
Dialysable Fulvic Acid	63	6
Fulvic Acid	11	9
Humic Acid	26	85

Considerable effort has been devoted to optimisation of the gel permeation technique. In particular, Fractogel media (Merck, Darmstadt, W. Germany) are superior in several respects to the Sephadex (Pharmacia Ltd., Milton Keynes, UK) media originally used (Livens et al, 1987). They are more robust, permitting the use of higher pressures and faster flow rates and reducing the risk of compaction of the gel bed. Unfortunately, there appears to be extensive interaction between humic material and the Fractogel media which causes poor resolution and substantial sample losses by adsorption to the gel bed.

In preliminary experiments (Livens et al., 1987), adequate resolution of the humic acid fraction was achieved using a column of Sephadex G-150, 3 cm diameter and 50 cm in height. Resolution of this fraction has been improved by using G-100, a gel with a slightly smaller pore size, and reduction of the column height to 30 cm. These changes actually permit separation of the humic acid into two discrete fractions which constitute 60% and 40% of the humic acid respectively.

A number of techniques are available for the determination of different 'classes' of organic compound in soil organic matter (Stevenson, 1982). One which has been applied to radionuclide speciation is Soxhlet extraction using a series of organic solvents (Vos et al., 1983). As a first step, this technique has been applied to the humic acid fraction, using solvents of increasing polarity, and gives the following results (Table 2):

Table 2. Solubility of humic-complexed Pu in solvents of increasing polarity

Solvent	% Wt lost	Pu-239, 240(mBq)
Hexane	2	1.1
Diethyl ether	1	0.2
Ethyl acetate	1	0.6
Methanol	32	122
Acetic acid	1	9.9

References

Livens, F.R. & Baxter, M.S. (1988). Chemical associations of artificial radionuclides in Cumbrian soils. J. Environ. Radioactivity, **7**, 75-86.
Livens, F.R., Baxter, M.S. & Allen, S.E. (1987). Association of plutonium with soil organic matter. Soil Sci., **144**, 24-28.
Stevenson, F.J. (1982). Humus chemistry, genesis, composition, reactions. Wiley, London.
Vos, H.A., Williams, G.A. & Cooper, M.B. (1983). Speciation of radionuclides in sediments and soils. Part II Studies with a sequential organic extraction procedure. Australian Radiation Laboratory Report ARL/TR058.

IV. Objectives for the next reporting period:

1) Optimisation of the gel permeation technique for fulvic acid to provide the same quality of sample resolution that has been achieved for the humic fraction;
2) Characterisation of each humic and fulvic fraction, including functional group determinations (Peachey & Williams, 1988).
3) Continuation of the Soxhlet extraction experiments.
4) Publication in the scientific literature.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Delta Institute for
Hydrobiological Research
Vierstraat 28
NL-4401 EA Yerseke

Contract no.: BI6-B-191-NL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Heip
Delta Institute
Vierstraat 28
NL-4401 EA Yerseke

Telephone number: 1131/1920

Title of the research contract:

Transfer processes and modelling of plutonium species and gamma emitters in the Scheldt estuary ; redox and organic speciation in relation to aqueous and particulate fractionation.

List of projects:

1. Transfer processes and modelling of plutonium species and gamma emitters in the Scheldt estuary ; redox and organic speciation in relation to aqueous and particulate fractionation.

Title of the project no.: B16-B-191 NL

Transfer processes and modelling of plutonium species and gamma emitters in the Scheldt estuary; redox and organic speciation in relation to aqueous and particulate fractionation

Head(s) of project:

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Scientific staff:

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I. Objectives of the project:

Study of dissolved and particulate ^{238}Pu and ^{239}Pu, ^{240} and gamma-emitters (Co-60, Cs137...) in the Western Scheldt area;
Determination of Kd's as a function of major physico-chemical parameters such as dissolved oxygen, pH, Eh, salinity, DOC and POC.
Study of redox partitioning of dissolved plutonium (III + IV) and (V + VI) at selected locations of the Western Scheldt.
Study of the role of sediment particles as sorption substrate.

II. Objectives for the reporting period:

Field and experimental work to determine origin and fate of the studied nuclides. 1. Field measurements along the Western Scheldt estuary (Gent to Vlissingen): a) Measurement of major physico-chemical parameters: distribution of salinity, temperature, oxygen, total suspended matter, particle size and organic content of suspended matter, dissolved organic matter including amino acids, N- and P-nutrients, concentration of plankton and biological activity. b) Measurements of total Pu in suspended matter and water, redox Pu speciation in water and gamma emitters.

2. Experimental work: a) Determination of adsorption kinetics of plutonium and gamma emitters under oxic and anoxic conditions. b) Determination of the base exchange capacity of selected sediment samples.

III. Progress achieved:

Three cruises have been carried out along the Western Scheldt with the R/V Luctor from the Delta Institute in March, August and November 1988. During these cruises the major physico-chemical parameters have been determined. Surface water samples (200 l) and suspended matter were collected in a wide range of salinities from 2 - 32 ‰ . The separation of the particulate phase and coprecipitation of dissolved plutonium were made using the same procedures as in 1987. For adsorption experiments Scheldt water was spiked with ^{237}Pu (III + IV) or ^{237}Pu (V + VI) and maintained under oxic or anoxic conditions. Filtration of aliquot samples was performed as a function of time for subsequent gamma counting and K_D determination. Analyses of Co, Ni, Al and suspended matter were performed.

Normalized concentrations in the river samples beyond the tidal region and in the estuary do not differ much from previous observations and show a notable decrease from the mouth to the central part of the estuary. High $^{238}Pu/^{239+240}Pu$ ratios have been detected in the Bovenschelde and possibly in the Albertkanaal. These data confirm that the predominant source of $^{239+240}Pu$ is the North Sea.

The high activity ratios probably result from two different causes:
- the "Nete-anomaly" (also found with gamma-emitters) probably reflects reworking of contaminated sediments and/or nuclear effluents origination from the Mol/Eurochimic zones. This would explain the highest $^{238}Pu/^{239+240}Pu$ ratios in the Rupel area and confirms the existence of an industrial ^{238}Pu-source (1979-1984 study).
- high activity ratios (in the range 0.07-0.24) in the Boven Scheldt and Dijle and possibly the Albertkanaal are likely to represent a mixture of Chernobyl and pre-Chernobyl sediments. It is not understood at the moment why this ratio reached a maximum only 7 months after Chernobyl in the Dijle and 18 months in the Bovenscheldt.

Results for dissolved species are similar to the 1987 results. The difference with the much higher 1986 results is thus confirmed. The discrepancy has been attributed to the use of filters with different porosity. However, in December 1986 the pH was significantly lower in the estuarine zone than during the following 1987 surveys and even small pH changes may to a certain extent control Pu adsorption.

The $^{137}Cs/Al$ ratio in samples collected in 1986-1988 in rivers is higher by one order of magnitude than in the pre-Chernobyl samples. This large enrichment is still observed in the upper estuary but the ratio decreases to pre-Chernobyl values near the mouth. $^{134}Cs/^{137}Cs$ ratios reached maximum values in June 1986 (0.43 in the Bovenscheldt and 0.47 in the Dijle). Later measurements at Gent showed lower ratios decreasing with the ^{134}Cs half-life till October 1987. In the Nete sample the ratio is only 0.30.

A nearly linear decrease of the activity ratio with distance in the estuary was observed in 1986 and 1987. These data are very different from those collected in 1979-1984 which show an introduction into the estuary of ^{137}Cs associated to suspended matter from marine origin and a nearly constant ^{134}Cs background (activity ratio of about 0.06) probably due to Doel effluents. This difference obviously results from the Chernobyl input. When the ^{137}Cs due to Chernobyl is substracted most of the non-Chernobyl component falls into the range of the 1979-1984 measurements.

It would be interesting to model the ^{134}Cs ^{137}Cs ratio which decreases so regularly in

the estuary and essentially reflects the propagation of a terrigenous signal in the suspended matter stock. This would help understanding why the ^{137}Cs-content of the sediments near the mouth remained unaffected by the Chernobyl event. The development of a simple model should be envisaged; it could provide useful information and generalisation concerning the response of this estuarine system to an atmospheric poollutant input.

Only preliminary results on the adsorption experiments are available: oxic plutonium is more readily absorbed on particles than reduced plutonium but the difference decreases with time. Cobalt and Manganese are more soluble under anoxic conditions than expected. In one experiment in oxic conditions part of Pu, Cd and Mn were released from the particles: this mobilisation might be related to an important algal bloom.

Since Co is one of the major radioactive elements released by the nuclear power plant in Doel, special attention was devoted to the development of an analytical method able to describe its speciation in the estuary. The method is based on cathodic stripping voltametry and enables us to distinguish between free dissolved inorganic cobalt, organically complexed dissolved cobalt and easily available particulate cobalt. Several longitudinal profiles of these species in the Western Scheldt have been collected. They all show a systematic maximum of total Co in the area of Doel with a predominant contribution of the dissolved inorganic fraction. The total concentration observed at a given position is strongly dependent on the water discharge suggesting a source term of constant strength.

The rapid transfer of many contaminants form the dissolved to the particulate phase lead us to consider the possibility of developing a rapid method for the quantitative analysis of suspended matter in aquatic systems. The first part of the study concerned the direct analysis of small quantities (a few mg) of suspended matter collected on filter and resuspended in viscous slurries by atomic absorption. This method tested with certified international standards appeared to be very effective. The second aspect concerns the quantitative analysis of major elements in the same suspension. This information is essential to understand the origin and the behaviour of contaminants in the estuary. A method based on X-ray fluorescence is currently being developed.

Results from the sedimentological survey in December 1986 with the Luctor and the Jan Verwey from the Delta Institute are now available. Between Bath and Dendermonde a turbidity maximum with concentrations in suspended matter of 800 mg/l near the bottom was found. In the freshwater zone up to Gent the concentration of suspended matter was 40-60 mg/l and in the estuary downstream from Bath 30-55 mg/l. Relatively high numbers of particles larger than 16 µm exist in the freshwater part but not in the estuarine part. However, this may be an artifact due to a smaller stability of flocs in more saline waters.

The concentration of organic matter gradually decreased from 20-40 % in the freshwater part to 14-20 % in the estuary, with some exceptions. ETS measurements of biological activity show a regular decrease in the freshwater from Gent (22-30 µmol O_2/l/h) to Antwerpen (8-10 µmol O_2/l/h) which continues in the estuary. From Bath to Vlissingen the biological activity was very low (less than 2 µmol O_2/l/h. The lack of correlation between organic content, biological activity (mainly bacterial metabolism at this time of the year) and particle size indicates that particle concentration is not important. The base exchange capacity of sediments was found to be between 0.65 and 7.28 mval.

IV. Objectives for the next reporting period:

- In situ measurements of particle size and shape along the Schelde river with an in situ suspension camera system recently developed at NIOZ (Texel) with additional measurement of major physico-chemical parameters
- Scanning microprobe analyses of suspended particles to determine the origin of the organic matter
- Synthesis and publication of data obtained during the previous cruises including simple modelling of isotope distribution in the Scheldt estuary

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

- Publication of the results is planned for 1989-1990

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB- London SW1Y 4QP

Contract no.: BI6-B-044-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr J.S. Hislop
Env.& Med. Sciences Div.
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Didcot
GB- Oxon OX11 ORA

Telephone number: 0235/24.141

Title of the research contract:

The remobilisation and transport of actinides from sediment deposits in West Cumbria.

List of projects:

1. Further studies of the mechanism of remobilisation of actinides in the Ravenglass estuary and estimation of the total deposit of actinide in the estuary.
2. The source of actinide-bearing sediments in the surf zone in West Cumbria.

Title of the project no.:

(1) Further studies of the mechanism of remobilisation of actinides in the Ravenglass estuary and the total deposit of actinides in the estuary.

Head(s) of project:

Dr J S Hislop
Environmental & Medical Sciences Division,
Harwell Laboratory, Didcot, Oxon OX11 0RA

Scientific staff:

Mr P J Burton
Dr R C Carpenter
Miss L P Yarnold

I. Objectives of the project:

This project was designed to study the environmental and radiological consequences of the large accummulation of actinides in the intertidal sediment deposits of the Ravenglass estuary, 10 km SE of the Sellafield reprocessing plant in Cumbria. Preliminary studies had shown that plutonium and americium were remobilised into solution from these sediments at low tide, leading to net losses to the sea on the ebb tide. Investigations of the magnitude of this effect and the mechanisms controlling it were the main aims of the work. These studies are a continuation of two previous contracts (B10-D-334-81-UK(H) and B10-B-561-84-UK).

II. Objectives for the reporting period:

(a) To extend and confirm, under a range of conditions, the observations of net losses of actinides from the estuary, due mainly to material adsorbed on suspended particulates.

(b) To establish what proportion of the total amounts of actinides in the estuary's sediment deposits are lost by this means per tide by an estimate of the inventory of the estuary involving a large programme of sediment coring.

III. Progress achieved:

METHODOLOGY

(a) Tidal Balance Studies

To measure the amounts of plutonium and americium entering and leaving the estuary over complete tidal cycles; mid-depth, mid-stream samples were taken at 30 min intervals from a boat moored near the mouth of the estuary (1-4). Measurements were made of flow, depth, sediment load, salinity and actinides in the soluble (<0.22 μm) and suspended particulate phases. On one occasion, to economise on analysis costs, composite bulks for the flood and ebb tides were made up from the individual samples in proportion to their relative contribution to the total volume. In the final two experiments, sampling was done remotely from the shore via pipes attached at different heights below a large moored buoy. This allowed sampling in sea conditions too rough for safe working from an inflatable boat.

(b) Actinide Inventory

The estuary contains a variety of sediment types such as sand, consolidated saltmarsh, shingle and fluid mud. Sand cores were taken using 25 mm (i.d.) PVC tubing (5), saltmarshes were sampled with 80 mm (i.d.) steel tubes and shingle areas were cored by digging 15 x 15 cm square sections out with a spade. These are all standard techniques, but a method for sampling mud had to be developed as these areas can be too soft to walk on. Sampling with 50 mm (i.d.) perspex tube from a boat floating in shallow water on a rising tide overcame this difficulty. A constant pressure in the tube above the core was maintained by filling with water and capping with a tight rubber bung, allowing extraction from the mud without the sample being sucked out.

Core sampling of the entire estuary was carried out in 1985-86. Over 250 individual cores were taken and bulked into 44 larger samples representing areas of similar deposit. These were air dried, ground, thoroughly homogenised and analysed for Pu and Am by standard radiochemical techniques followed by α-spectrometry (4).

RESULTS

Table 1 summarises the results of the activity balances. In all the experiments the amounts of water and salt entering and leaving the estuary agreed to within 10%, indicating no sampling bias. However, there were net imbalances in the actinides, especially in the suspended particulate phase. In two experiments (cycles E & G) there were net losses of actinides, whilst in cycles F, H and J, when there was net sediment accumulation in the estuary, there was a net gain of activity by the estuary.

Figs 1-8 give more detail for two contrasting cycles, including data on changes in suspended sediment load and its actinide concentration.

The results of the core sampling survey are summarised in Table 2. A report is being prepared for publication in a scientific journal which will include the full data set from this survey which was too extensive for the present summary.

DISCUSSION

The tidal balance work in this study period has confirmed that the Ravenglass estuary can act as a source of actinides to the sea. However, it has also shown, for the first time, that under certain conditions the reverse can occur. This happens when strong tides and storms at sea lead to mobilisation of sand and sediment from the sea bed and transport into the estuary on the flood tide. The estuary is shallow and lies in the lea of high dunes, so over high tide and on the ebb this material deposits out. In all the cycles where net actinide accumulation occurred it was accompanied by sediment deposition. This does not mean that some remobilisation from deposited material does not occur on these tides, but the balance is in favour of accummulation.

The study has concluded that the mechanism of actinide remobilisation involves desorption into solution at low tide, when pH and salinity are low, with k_d values of $\sim 10^4$. This material is then thought to readsorb onto less active suspended material brought in by the flood tide with k_d values of 10^5 - 10^6, and some of it flushes out to sea in association with suspended particulates on the ebb. These conclusions were reached

partly from the results of laboratory studies (6).

Table 2 shows that a large proportion of the actinides in the sediments are in the River Esk. Levels are generally highest in the saltmarshes with the peak level of $^{239+240}Pu$ found being >5000 Bq kg^{-1} dry weight. Analysis of sectioned cores showed that the activity is confined to the top 30 cm of the saltmarshes but can penetrate down to 60 cm or more in sandy areas.

In order to assess the amount of this deposited activity that is available for remobilisation and the timescales involved, a number of factors need to be taken into account. These include radionuclide distribution with depth, movements of channels and banks with time, sedimentation and scouring rates at different locations, ingrowth of ^{241}Am from ^{241}Pu, bioturbation and radionuclide speciation effects. To resolve this complex situation a computer modelling approach to radionuclide behaviour and ultimately to dose prediction has recently begun. In the meantime, the present results suggest that an average of in the order of 100 MBq of $^{239+240}Pu$ may be lost from the estuary per tide under most conditions with similar net gains on a few tides per year in suitable stormy conditions. Thus 60 GBq may be the current annual loss to the sea. This represents a few percent of the inventory, although as indicated above not all the material deposited may have the potential for remobilisation.

Thus it may be concluded that despite large recent decreases in actinide discharge from Sellafield to the sea, estuaries such as that at Ravenglass will continue to have relatively high levels in their surface sediment deposits and will have a radiological consequence for many years to come.

Table 1: $^{239+240}Pu$ & ^{241}Am entering and leaving the Ravenglass estuary during tidal cycles

CYCLE & DATE	$^{239+240}$ Pu (MBq)				^{241}Am (MBq)			
	SOLUTION		SEDIMENT LOAD		SOLUTION		SEDIMENT LOAD	
	FLOOD	EBB	FLOOD	EBB	FLOOD	EBB	FLOOD	EBB
E: 24.6.85	44	56	160	270	4.4	5.2	220	350
F: 17.9.85	180	180	1900	1400	66	37	2200	1800
G: 5.9.86	140	170	1200	1400	14	16	1400	1600
H: 22.10.87	41	47	510	310	3.1	9.2	640	430
J: 15.11.87	67	64	3200	2700	17	26	4900	5300

Conditions in Estuary

Cycle E: Three days after spring tide, stiff W breeze, 5-7 ms^{-1}. River flow high due to several days of rain. Sediment loads quite high.

Cycle F: One day after spring tide. Stormy conditions on previous two days and in early part of this cycle. Wind dropped from <10 ms^{-1} to 3-4 ms^{-1} around high water. Sediment loads high on flood, but dropped on ebb. Net accumulation of sediment in estuary.

Cycle G: Two days before spring tide. Wind W 6-8 ms^{-1} throughout. Heavy swell after tide covered bar. Sediment loads high.

Cycle H: Two days before spring. SW 6-8 ms^{-1} on flood, 4 ms^{-1} on ebb. High river flow due to recent rain. Net sediment accumulation.

Cycle J: One day after neap. SW 2-4 ms^{-1} on flood, 6 ms^{-1} on ebb. Rough sea after winds on previous day. Sediment loads high. Net sediment accumulation.

Table 2: Inventory of actinides in the intertidal sediment deposits of the Ravenglass estuary (summary)

BRANCH	$(239+240)_{Pu}$ (GBq)	^{238}Pu (GBq)	^{241}Am (GBq)
ESK	920	193	1314
MITE	203	42	269
IRT	470	93	578
MOUTH	18	3	40
TOTAL (GBq)	1611	333	2202
TOTAL (TBq)	1.611	0.333	2.202

Fig 1. 5/9/86 TIDAL CYCLE.

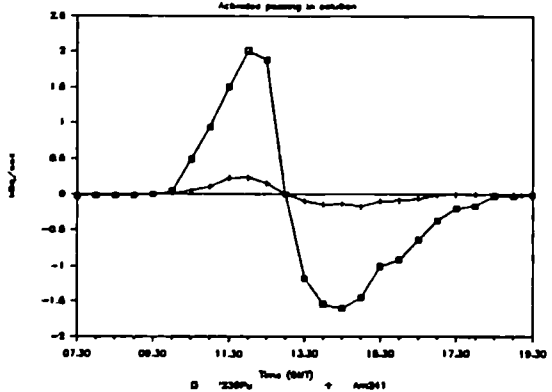


Fig 2. 5/9/86 TIDAL CYCLE.

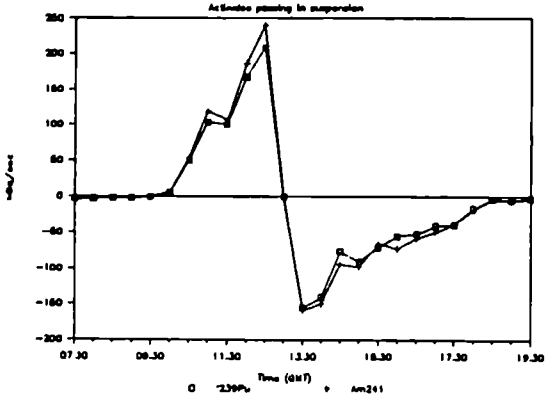


Fig 3. 5/9/86 TIDAL CYCLE.

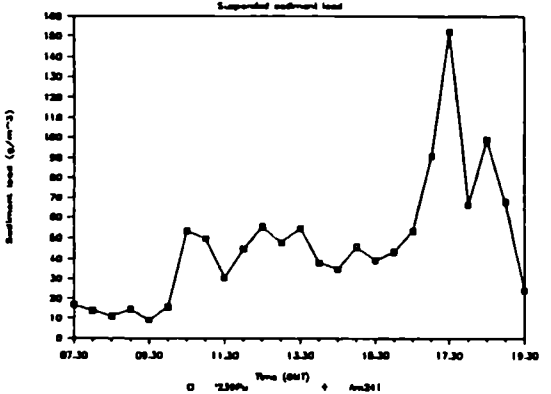


Fig 4. 5/9/86 TIDAL CYCLE.

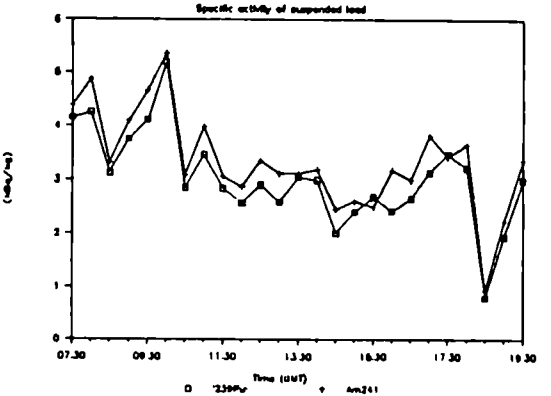


Fig 5. 15:11:87 TIDAL CYCLE

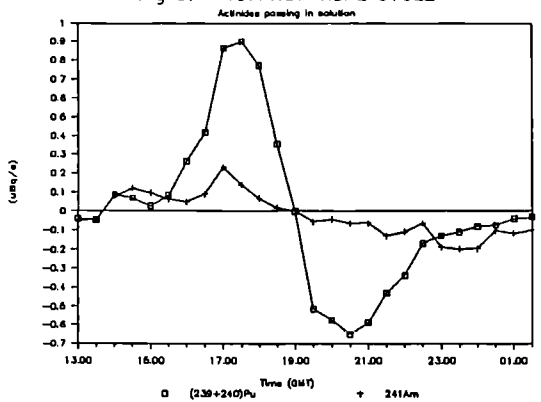


Fig 6. 15:11:87 TIDAL CYCLE

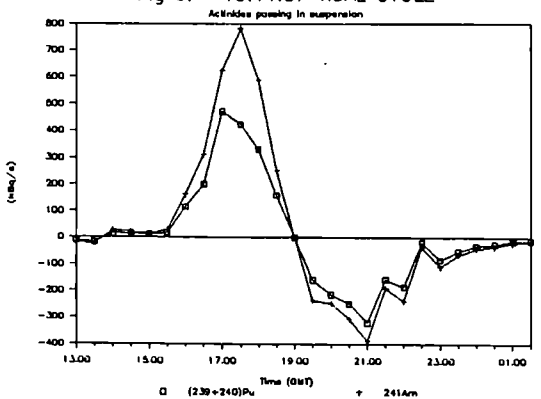


Fig 7. 15:11:87 TIDAL CYCLE

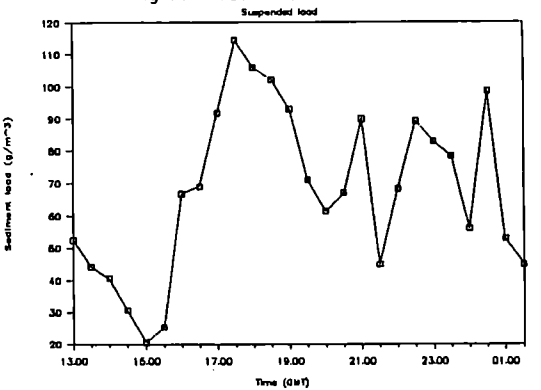
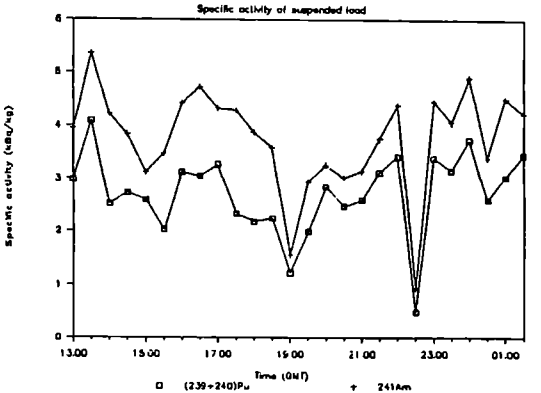


Fig 8. 15:11:87 TIDAL CYCLE



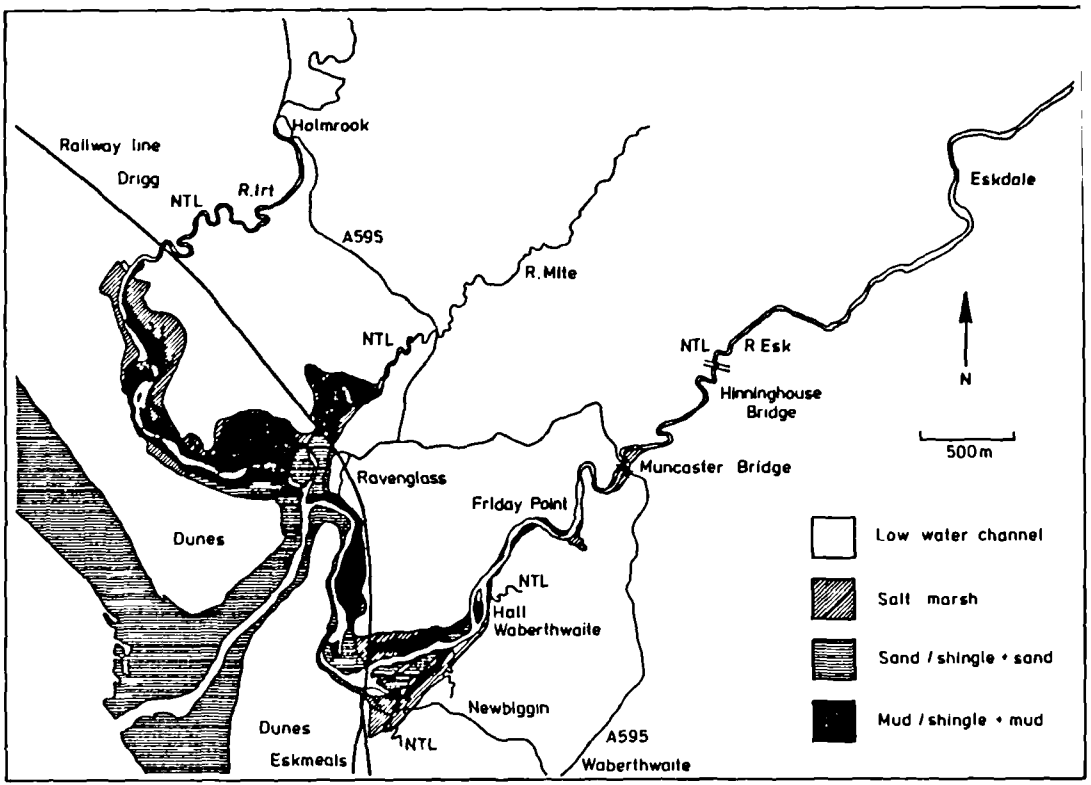


FIG. 9 SEDIMENT DEPOSITS IN THE RAVENGLASS ESTUARY

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None

V. Publications:

1. Morgan, A., Eakins, J.D., Burton, P.J., Humphreys, D.G. and Lally, A.E.. Euratom Radiation Protection Programme Progress Report 1980-84 Vol. 1. EUR 9733 DE/EN/FR 644-648 (1985).

2. Eakins, J.D., Burton, P.J., Humphreys, D.G. and Lally, A.E.. Proc Seminar on The Behaviour of Radionuclides in Estuaries, Renesse, Netherlands, 1984, CEC Seminar/380/85-EN (1985).

3. Burton, P.J., Eakins, J.D. and Lally, A.E.. Proc Seminar on Application of Distribution Coefficients to Radiological Assessment Models, Lonvain-la-Neuve, Belgium, 1985, (1986).

4. Burton, P.J. and Yarnold, L.P.. Science Total Env., 69 (1988) 239-260.

5. Eakins, J.D., Morgan, A., Baston, G.M.N., Pratley, F.A., Yarnold, L.P. and Burton, P.J.. AERE R-12061 (1987).

6. Burton, P.J.. Science Total Env., 52 (1986) 123-145.

Title of the project no.:
The source of actinide-bearing sediments in the surf zone in West Cumbria.

Head(s) of project:
Dr J S Hislop
Environmental & Medical Sciences Division,
Harwell Laboratory, Didcot, Oxon OX11 0RA

Scientific staff:
Mr P J Burton
Miss L P Yarnold
Dr R C Carpenter

I. Objectives of the project:

This project was designed to investigate the origin of actinide-bearing sediments. The materials of interest included seaspray particulate, sediment in bulk seawater and marine and estuarine deposits.

In addition, the investigation was planned to study an apparent seasonal variation in actinide enrichment factors observed by Cambray[1] from air samplers sited at Eskmeals in West Cumbria, approximately 14 km south of the Sellafield reprocessing plant.

II. Objectives for the reporting period:

III. Progress achieved:

METHODOLOGY

Collection and Preparation of the Samples

Airborne Particulate Material

Muslin screen collectors were used to sample the particulates in seaspray[2]. These consisted of strips of muslin 5 m long and 1 m wide mounted vertically on supports. They were erected so that the longer sides were 1 m above the ground.

Muslin screen collectors were deployed on four beaches in West Cumbria (Eskmeals, Drigg, Seamills and St Bees) in the spring and autumn of 1985. Sampling conditions of strong onshore wind (velocities ~ 10 m sec^{-1}) were chosen when possible.

The screens were exposed for an average of 3 hours, after which time the discolouration of the screens due to the particulate material collected could be distinctly observed.

The screens were then repeatedly washed in solutions of artificial seawater and the particulates collected. The supernates and particulates were then stored at -20°C to prevent any degradation of the samples until analysis was carried out.

Bulk Seawater Samples

Samples of seawater were also collected at Seamills and Drigg Beaches, from the surf zone.

The samples were allowed to stand for one minute in order to let the larger sand particles settle out prior to filtering through a membrane filter (Millipore g.s. pore size 0.22 μm).

The sediment was then washed from the millipore filters with artificial seawater and stored at -20°C.

Sediment Samples

Sediment samples were collected from the Ravenglass and Duddon estuaries, the Solway Firth and the mud bank off of St Bees head in the Irish Sea. These samples were air dried until required for analysis.

In order to standardise the determinations prior to passing through the extraction procedure, the particulate material from all the samples was separated from any large sand particles by an agitation and settlement procedure. A determination of the total organic material was also carried out on some of the samples.

Preliminary Tests on the Extraction Procedure

To evaluate the extraction procedure reported by Tessier et al[3] a series of trial experiments was carried out both on the individual fractions of the scheme and the scheme as a whole.

The results obtained demonstrated that the procedure was reproducible within ± 1%.

Extraction and Analysis

Although this particular sequential extraction procedure cannot totally discriminate between the bound forms of plutonium and americium, the technique does enable an acceptable comparison to be made between the samples.

Due to the activity levels of the samples, it was possible to carry out the extractions on the same size aliquots (1 g) as used by Tessier. Therefore no alteration of reagent volumes was necessary.

The extraction procedure can be summarised as follows:

a) exchangeable: shaken with 1M magnesium chloride at pH 7.0 at room temperature for 1 hour.

b) carbonate: shaken with 1M sodium acetate and acetic acid at pH 5.0 at room temperature for 6 hours.

c) iron and manganese oxides: digested with hydroxylamine hydrochloride

and acetic acid at pH 2.0 and 96°C for 6 hours.

d) organic fraction: digested with 0.02M nitric acid and hydrogen peroxide at pH 2.0 and 85°C for 5 hours, then shaken with 3.2M ammonium acetate in nitric acid at room temperature for 30 minutes.

e) residual fraction: total dissolution with nitric acid and hydrofluoric acid.

After each extraction the samples were centrifuged at 10,000 rpm for 30 minutes and the supernate transferred to a beaker. The residue was then washed with water, re-centrifuged and the washings added to the bulk supernate.

The analysis of each extraction stage was carried out by established radiochemical techniques and alpha spectrometry.

RESULTS

Results from determinations of total organic/inorganic ratios of seaspray particulates are given in Table 1. Tables 2-5 give the distributions of $^{239+240}Pu$ and ^{241}Am in sequential extractions carried out on the seaspray particulates, surf zone suspended sediments and surface sediment deposits.

DISCUSSION

Organic/Inorganic Ratios

The total organic/inorganic ratios (Table 1) show a consistent increase in the organic content of airborne particulate collected in September compared to that collected in March.

The mean results for plutonium in monthly air samples collected at Eskmeals between 1978 and 1984 indicate a seasonal effect[5]. The plutonium in air contents are low in the winter months and rise to a maximum in September. This peak corresponds with an increase in sea temperatures and possibly indicates the presence of plankton blooms. The increased organic content of the airborne particulates collected in September may reflect an increase in availability of plant material in

inshore waters for adsorption of actinides.

Sources of actinide-bearing sediments in the surf zone

As a result of possible errors in sampling and extraction and the uncertainties associated with the sample counting, only general trends are examined here. Also as the amount of activity collected on the muslin screens depends on parameters such as site, exposure time, wind speed and sea conditions, when comparing results percentages have been used rather than the absolute values.

The results indicate that the majority of the plutonium is associated with the Fe/Mn oxides, organic and residual phases, with very little being ion exchangeable or in humic substances. This corresponds with results from other studies carried out on estuarine sediments from Buzzards Bay, USA[4] and the Ravenglass estuary in West Cumbria[5]. The distribution of americium differs somewhat, in that the majority is associated with the carbonate, Fe/Mn oxide and organic fractions. This is in agreement with results obtained by Alberts et al[6] in freshwater studies of the Savannah river plant, South Carolina, where there was an increase in ^{241}Am relative to $^{239+240}Pu$ in the humic acid fraction of sediment samples.

Tables 2 and 4 indicate that plutonium associated with particulate material in the surf zone at Seamills and in seaspray at Eskmeals have similar actinide distributions to material from the Ravenglass estuary. Tables 3 and 5 show a similar pattern for ^{241}Am. Actinides are lost from the estuary on the ebb tide in association with suspended material which may be transported to the intertidal region at nearby beaches, forming part of particulate content of the seaspray. In this region only 0.1% by weight of the intertidal deposits outside the estuary are <10 μm in size[8], therefore fine material in the seaspray must be continually replaced in the surf zone from outside, rather than being produced from the action of waves on the beach. Table 4 also demonstrates similar actinide distributions between surf zone seawater suspended particulates from Seamills, samples from the Duddon estuary and the offshore mud bank.

This study seems to indicate that these areas of muddy deposits may be

the source of some of the fine actinide-bearing sediments in the seaspray.

Seasonal comparison of particulate material on muslin screens

There was an increase in the total Pu and Am in the autumn compared to the spring in all 5 phases (Tables 2 and 3).

For plutonium the greatest percentage increase was in the amount associated with the organic phase. In general the distributions for americium did not change as markedly.

The behaviour of plutonium might be explained by the fact that micro-organisms such as algae and bacteria take up the actinides early on in the year and when they decay in the autumn form fine particulates enhanced in organically-bound Pu. These particulates become available for scavenging by rising bubbles in the surf zone followed by injection into the air as spray.

Alternatively this enhanced effect may be due to uptake by various micro-organisms, corresponding to an algal bloom occuring in the autumn, which may be suspended in seaspray and blown inland.

Further studies are being carried out into the role of organic material in seaspray production. This involves measuring seasonal variations in bacterial populations and in surfactant levels.

This work forms one section of a report currently in preparation on all aspects of actinide enrichment in seaspray in West Cumbria.

Table 1. Total Organic to Inorganic Ratios for Three Muslin Screen Collections

Sampling Site	March	September
Eskmeals	1.22	2.0
Seamills/St Bees	1.25	2.3
Drigg	1.40	2.6

Table 2 Distribution of $^{239+240}Pu$ in seaspray particulate

Sample	Muslin Screen Eskmeals June Bq kg^{-1}	%	Muslin Screen Eskmeals September Bq kg^{-1}	%	Muslin Screen Drigg March Bq kg^{-1}	%	Muslin Screen Drigg September Bq kg^{-1}	%	Muslin Screen St Bees/Seamills June Bq kg^{-1}	%	Muslin Screen St Bees/Seamills September Bq kg^{-1}	%
Exchangeable	2	0.2	1	0.1	<1	0.1	1	0.1	1	0.1	2	0.1
Carbonate	27	2.5	30	2.5	8	1.3	25	0.8	11	1.0	36	1.0
Fe/Mn oxide	403	37.1	315	26.7	168	26.0	383	11.7	267	23.2	844	23.4
Organic	214	19.7	370	31.4	49	7.6	1556	48.0	105	9.2	693	19.3
Residual	441	40.5	463	39.3	421	65.0	1275	39.4	762	66.5	2028	56.2
Total	1087	100	1179	100	646	100	3240	100	1146	100	3603	100

Table 3 Distribution of ^{241}Am in seaspray particulate

Sample	Muslin Screen Eskmeals June Bq kg^{-1}	%	Muslin Screen Eskmeals September Bq kg^{-1}	%	Muslin Screen Drigg March Bq kg^{-1}	%	Muslin Screen Drigg September Bq kg^{-1}	%	Muslin Screen St Bees/Seamills June Bq kg^{-1}	%	Muslin Screen St Bees/Seamills September Bq kg^{-1}	%
Exchangeable	2	0.2	1	0.1	8	1.0	2	0.1	2	0.2	2	0.1
Carbonate	250	19.2	308	22.1	199	24.2	451	11.7	168	12.5	632	13.8
Fe/Mn oxide	619	47.4	614	44.1	414	50.3	1580	40.4	649	48.1	2172	47.2
Organic	255	19.5	253	18.1	122	14.8	1239	31.3	344	25.5	1286	28.0
Residual	179	13.7	220	15.6	80	9.7	625	16.5	185	13.7	503	10.9
Total	1305	100	1396	100	823	100	3897	100	1348	100	4595	100

Table 4 Distribution of $^{239+240}Pu$ in surf zone suspended sediment and surface deposits

Fraction	Activity, Bq kg^{-1} (dry weight); (% of total)							
	Surf zone sediment		Surface sediment					
	Seamills September 1985		Ravenglass Estuary June 1984		Duddon Estuary September 1986		Offshore Mudbank April 1985	
	Bq kg^{-1}	%	Bq kg^{-1}	%	Bq kg^{-1}	%	Bq kg^{-1}	%
Exchangeable	1	0.2	2	0.1	1	0.4	1	0.2
Carbonate	22	3.5	84	2.7	12	5.9	24	3.9
Fe/Mn oxides	247	39.0	927	30.7	85	42.4	200	32.0
Organic	151	23.4	640	21.2	59	29.4	103	16.7
Residual	216	33.9	1371	45.3	44	21.9	295	47.2
Totals	637	100	3024	100	201	100	623	100

Table 5 Distribution of ^{241}Am in surf zone suspended sediment and surface deposits

Fraction	Activity, Bq kg^{-1} (dry weight); (% of total)							
	Surf zone sediment		Surface sediment					
	Seamills September 1985		Ravenglass Estuary June 1984		Duddon Estuary September 1986		Offshore Mudbank April 1985	
	Bq kg^{-1}	%	Bq kg^{-1}	%	Bq kg^{-1}	%	Bq kg^{-1}	%
Exchangeable	2	0.2	2	0.1	2	1.2	<1	0.1
Carbonate	227	32.0	685	24.7	68	33.6	313	43.6
Fe/Mn oxides	326	45.8	1235	44.6	95	47.2	232	32.3
Organic	106	14.3	562	20.3	28	13.6	128	17.9
Residual	54	7.7	288	10.4	9	4.4	44	6.1
Totals	715	100	2772	100	202	100	718	100

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

1. R S Cambray, N J Pattenden and K Playford. AERE-R12617 (1988).

2. J D Eakins, A E Lally, P J Burton, D R Kilworth and F A Pratley. AERE-R10127.

3. A Tessier, P G C Campbell and M Bison. Anal Chem vol 51, No 7, June 1979, p844-851.

4. Alberts, J J, Muller, R N and Orlandini, K A (1976) ANL-76-88.

5. S R Aston and D A Stammers (1981) Nature 289, 581-582.

6. J J Alberts, J G Halverston and K A Orlandini. Journal of Environmental Radioactivity, vol 3, No 4, 1986.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Biologische Anstalt Helgoland
Notkestrasse 31
D-2000 Hamburg 52

Contract no.: BI6-B-039-D

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M. Hoppenheit
Laboratorium Sülldorf
Biologische Anstalt Helgoland
Wüstland 2
D-2000 Hamburg 55

Telephone number: 040/87.10.26

Title of the research contract:

Speciation and availability of Am in tidal water.

List of projects:

1. Reexamination of the concept 'concentration factor' for actinides and redefinition of the term taking into consideration the physical-chemical states of these radioelements.

Title of the project no.: 1

Reexamination of the concept 'concentration factor' for actinides and redefinition of the term taking into consideration the physical-chemical states of radioelements.

Head(s) of project:

M. Hoppenheit

Scientific staff:

H. Herrmann

I. Objectives of the project:

Transport and bioavailability of actinides are expected to be influenced by strong interaction with particulate matter. Suspended matter is of predominant importance, especially in tidal areas and estuaries. Investigations simulating the highly fluctuating chemical and physical conditions in coastal waters should provide more insight into the chemical processes influencing speciation and bioavailability of ^{241}Am.

II. Objectives for the reporting period:

The influence of the following factors on the bioavailability and speciation of Am in brackish waters was investigated: (1) the competition with seawater ions (changes in the Ca^{2+}-concentration), (2) the association with the anorganic constituents of the sediment (kaolin-admixture) and (3) the complexation by DOC (addition of citric acid; changes in the concentration of organic metabolic products by varying the time during which the animals stay in the medium). Experiments on the uptake of Am via the gills were initiated.

III. Progress achieved:

1. Methodology

The tests were carried out as batch experiments on adult males of the euryhaline amphipod Gammarus duebeni duebeni. Brackish water of $S \times 10^3 = 10$ i.e. 1.1 were used, the concentration of Am amounted to 0.6×10^{-9} mol x dm^{-3}, and that of purified kaolin or citric acid to 100 mg x dm^{-3}; by adding 480.5 mg x dm^{-3} $CaCl_2 \times 4H_2O$, the natural Ca-content of the brackish water $S \times 10^3 = 1.1$ was increased to that of seawater $S \times 10^3 = 10$. In experiments where the medium was changed daily, the pH value in every vessel was set daily over the course of one week (i.e. 5x), before starting the tests. 10 ml of the batch solutions were filtered through cellulose nitrate filters. The gill experiments were carried out on the isolated gills of Carcinus maenas in brackish water of $S \times 10^3 = 15$/pH 8 or pH 6.

2. Results

Increasing the natural Ca-content of seawater $S \times 10^3 = 1.1$ to that of $S \times 10^3 = 10$ results in Am-uptake that can be described by single-term exponetial functions both at pH 8 and pH 6. At the start of the experiments, these curves lie between those describing the Am-uptake in animals kept in seawater of $S \times 10^3 = 1.1$ and $S \times 10^3 = 10$; one week after the beginning of the experiments, however, they cross the latter and are higher. The addition of kaolin causes a decrease in the uptake of Am. The uptake in the medium $S \times 10^3 = 10$/pH 8/ kaolin displays the course of a single-term exponential function; that in the $S \times 10^3 = 1.1$/pH 8/kaolin medium can be described by a two-term exponential function. The complexation of Am with citric acid in the medium $S \times 10^3 = 1.1$/pH 8 leads to a more significant decrease in the uptake of Am. The remaining low Am-uptake is expressed by a curve displaying a single-term exponetial function. Experiments using aged $S \times 10^3 = 10$ medium, changed daily, lead to a drastic increase in Am-accumulation that rises almost linearly during the first week. This contrasts highly with the results gained in the experiments using unchanged, non-aged medium. The Am-uptake in the $S \times 10^3 = 10$/pH 8 medium is quite clearly higher than that in the $S \times 10^3 = 10$/pH 6 medium. The Am-adsorption to the walls of the beakers that occurs during the ageing process amounts to approximately 50% at pH 8; ca. 55% of the solute is filterable; at pH 6

about 100% of the Am remains in the solute, and about 75% of this is filterable. Preliminary experiments using isolated gills displayed no Am-transport through the membranes - neither at $S \times 10^3 = 15$/pH 8 nor at pH 6. On the other hand, Am-adsorption to the gills was very high.

3. Discussion

Most of the Am from seawater-solutions that is bound to the gammarids is adsorbed to the surface of the animals (i.e. to the exoskeleton, gills). The extent of this adsorption seems to depend in the main on the size and stability of the Am containing aggregates that have formed in the solution; the size of these aggregates, however, depends on the composition of the solution and the history of its development. The Am-aggregates have a strong tendency to adsorption to any surface, whether it be the walls of a container, animal body, or sediment partioles. On the addition of organic metabolic products, the Am-aggregates are broken up into organic complexes that are not adsorbed. The addition of Ca changes the formation of aggregates in the solution. Kaolin changes the surface area available for adsorption. The increase in Am-uptake observed during the course of the experiment at $S \times 10^3 = 1.1$/pH 8/ kaolin was a result of the fact that the animals used had been starved and after one week they began to consume the kaolin particles. When citric acid is added to the $S \times 10^3 = 1.1$/pH 8 medium, 90% of the Am is complexed, and as a citrate not adsorbed. A small amount of the in the strict sense bioavailable Am can be taken up through the surface of the skin, gills or gastro-intestinal tract. Apparently, no Am-transport takes place through the gill-membrane; changes in metabolism during moulting might bring about changes in this respect.

IV. Objectives for the next reporting period:

In the remaining available period the results achieved will be supplemented, especially by further experiments on Am-transport via the gills.

A synopsis of the results will be presented in the final report; publications will follow.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. Prof. Dr. O. Vanderborght, Radionuclide Metabolism Section, SCK/CEN, Mol, Belgium
2. Dr. D. Siebers, Biologische Anstalt Helgoland, Hamburg, GFR

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-B-041-D

Niedersächsisches Institut
für Radioökologie
Herrenhäuser Strasse 2
D-3000 Hannover 21

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr W.K.G. Kühn
Niedersächsisches Institut
für Radioökologie
Herrenhäuser Strasse 2
D-3000 Hannover 21

Telephone number: 762.26.05

Title of the research contract:

Transfer of radionuclides in the food chain.

List of projects:

1.Dynamic environmental cycling of HTO/HT/OBT (experimental studies and modelling).
2. Accumulation and long-term transfer of iodine-129 in the food chain, in human thyroid glands and in waters of waste deposits.

Title of the project no.: 1

Dynamic environmental cycling or HTO/HT/OBT
(Experimental studies and modeling)

A: HTO transfer in the atmosphere-soil system;
B: HT deposition, conversion and reemission as HTO.

Head(s) of project:

Dr. C. Bunnenberg

Scientific staff:

Dr. C. Bunnenberg
Dipl. Phys. M. Täschner
Dipl. Phys. J. Feinhals (until Sept. 88)
Dipl. Phys. B. Wiener (until Apr. 88)

I. Objectives of the project:

A. Transfer of HTO from atmosphere to soil under consideration of atmospheric and soil physical parameters affecting the deposition of HTO by condensation and molecular exchange. Investigations on exchange processes and isotopic effects during diffusion of HTO and H_2O in soils with regard to tritium accumulation in soil.

B. Studies on the physical conditions influencing the deposition velocity of HT on soils and conversion to HTO and OBT. Reemission of HTO from soils after HT-releases from nuclear facilities. Development of a mathematical model describing the cycling of tritium in the atmosphere-soil system.

II. Objectives for the reporting period:

A. Laboratory experiments to correlate deposition velocities of HTO and H_2O and the specific activity ratio to relevant meteorological and soil physical parameters. Extension of the mathematical model.

B. Continuation of the evaluation of the two HT field experiments. Laboratory experiments on the reemission of HTO with a soil column/wind tunnel arrangement to investigate single-parameter effects on the reemission rate.

III. Progress achieved:

A. HTO transfer in the atmosphere-soil system

1. Methodology

Theoretical considerations and results of earlier soil column experiments had shown that the transfer of H_2O and HTO from atmosphere to soil as a net effect of vapor exchange between the two compartments can be conveniently described on the basis of the deposition velocity concept. In a straight forward procedure it became clear that the fact, that the specific activity of the deposited moisture usually deviates from that of the atmospheric humidity by a factor k (specific activity ratio), may be attributed to differences in the deposition velocities v_{H_2O} and v_{HTO} of the two types of molecules, expressable exactly by the same factor:

$$\vec{v}_{HTO} = k \cdot \vec{v}_{H_2O}. \qquad (1)$$

As the deposition velocities are describing the fluxes and consequently the deposition yields, more soil column experiments have been carried out to study the dynamic behaviour of the components of the above relationship during a deposition process and their dependency on environmental parameters.

2. Results

Under the condition of constant HTO and H_2O contents in the ground level air, which are maintained in case of chronic releases and stable weather conditions when both types of molecules are resupplied by air movement, the deposition velocities of HTO and H_2O decrease drastically during the first hours of the deposition process. Because of proportionality this affects the deposition yield in the same manner. The most striking observation, which can also be verified by theoretical deductions, is the fact that k remains constant during the deposition process characterized by the defined conditions. This led to a thorough investigation on the dependency of k, and it was found that the specific activity ratio is solely depending on the absolute humidities of the

ground level air and of the soil air. Under isothermal conditions k can be derived from the respective relative humidities f_{air} and f_{soil} prevailing at the start of the HTO deposition process:

$$k = \frac{f_{air}}{f_{air} - f_{soil}}. \qquad (2)$$

3. Discussion

The experimental findings demonstrate that, apart from effects of changing meteorological conditions, the application of a standard HTO deposition velocity for purposes of deposition modeling may be highly misleading. The constancy of k during a deposition process under stable conditions simplifies predictions, as respective intervalls can be chosen, and the value of k can be derived from humidity data.

B. HT deposition, conversion and reemission as HTO

1. Methodology

The Canadian field experiment offered a lot of information on the tritium behaviour in the environment. Experimental evaluations of '88 concentrated on reemission from soil in the form of HTO. Measurements were performed at two locations downwind of the source at ground level and vertically up to 6 m at a distance of 32 m from the release point. Active sampling of HTO in the air was done with washing flasks (bubblers) filled with water, and for passive sampling of the air HTO Petri- dishes were exposed containing uncontaminated soil.

2. Results

The Canadian experiment showed a pronounced day-night cycle of the vertical air HTO profile. The most obvious difference between day (without rain) and night profiles is the lower air HTO concentration at all elevations at night, which is a consequence of lower reemission rates.

Another difference is the slope of the respective profiles. During night the decrease with height above ground is much steeper than at daytime. This is an effect of very low windspeeds at night, which results in less turbulent mixing of the atmosphere between ground level and higher elevations. Rainfall did not change the slope of the air HTO profile but reduced the absolute HTO concentrations, because the low-active rain dilluted the HTO content of the upper soil layer and, therefore, it slowed down the diffusional transport of the HTO molecules from deeper layers thus reducing the reemission rate. This could clearly be seen from the HTO profiles in soil.

Passive sampling of air HTO on uncontaminated soil samples confirmed the findings of our laboratory experiments on HTO deposition that reemission and deposition may occur at the same time depending on the orientation of the HTO concentration gradient at the air-soil interface.

3. Discussion

The overall reemission of HTO during some weeks after deposition of HT can be evaluated by a reemission rate of about 0.3 to 1 $\%\cdot h^{-1}$, which is quite consistent with the results of the French experiment. It is more difficult to describe the short-term effect on the HTO transport after short HT releases and conversion in soil. The immediate reemission rate might be higher than 10 $\%\cdot h^{-1}$, when the HTO of the top soil has not been washed down to deeper layers by precipitation and the turbulent transport is high.

For a more sophisticated description of the behaviour of HTO in the atmosphere-soil system laboratory experiments are going on at NIR. First runs with the soil column/wind tunnel set-up have been performed yielding preliminary information on short-term reemission rates and on resulting HTO profiles in soil.

IV. Objectives for the next reporting period:

A. Laboratory experiments to complete the parameterization of HTO deposition towards a mathematical model. Analysis and description of HTO profiles in soil as a result of deposition processes.

B. Laboratory experiments with the soil column/wind tunnel set-up to determine the effects of meteorological and soil physical parameters on HTO reemission rates.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

L. A. König, H. Schüttelkopf, S. Diabate, KFK, Karlsruhe, FRG
G. Ogram, F. Spencer, CFFTP, Ontario Hydro, Toronto, Canada
R. Brown, AECL, Chalk River, Canada
O. Edlund, Energiteknik, Studsvik, Sweden
H. Förstel, KFA, Jülich, FRG
Y. Belot, CEA/IPSN, Fontenay-aux Roses, France
J. van den Hoek, Agricultural University Wageningen, Netherland
R. Kirchmann, C. Vandecasteele, CEN/SCK, Mol, Belgium
H. Nogushi, JAERI, Tokai Reserch Establishment, 319-11 Japan

VI. Publications:

(1) Feinhals, J.; Bunnenberg, C.:
Laboratory investigations of HTO deposition to soils.
Fusion Technology 14 No. 2, Part 2B (1988) 1253 - 1257.

(2) Feinhals, J.:
HTO-Deposition durch Gasaustausch im System Atmosphäre-Boden.
Diss. U. Hannover (1988), NIR-Bericht NR. 1/88.

(3) Täschner, M.; Wiener, B.; Bunnenberg, C.:
HT dispersion and deposition in soil after experimental releases of tritiated hydrogen.
Fusion Technology 14 No. 2, Part 2B (1988) 1264 - 1269.

(4) Täschner, M.; Bunnenberg, C.:
Plume dispersion and deposition processes of tracer gas and aerosols in short-distance field experiments.
IV. International Symposium of Radioecology on "The Impact of Nuclear Origin Accidents on Environment", Cadarache, March 14 - 18, 1988.

(5) Wiener, B.; Täschner, M.; Bunnenberg, C.:
HTO reemission from soil after HT deposition and dose consequences of HT releases.
Fusion Technology 14 No. 2, Part 2B (1988) 1247 - 1252.

Title of the project no.: 2

Accumulation and long-term transfer of iodine-129 in the food chain, in human thyroid glands and in waters of waste deposits.

Head(s) of project:

Dr. J. Handl

Scientific staff:

Dr. J. Handl
Dr. D. Jakob

I. Objectives of the project:

Investigations on the long-term translocation of I-129 in soils, its transfer from soil to plant and along the food chain: pasture/cow/milk/thyroid gland.

Inventory of I-129 and I-127 in human thyroids to examine the long-term development of the isotopic ratio I-129/I-127 in the food chain. Studies on I-129 and I-127 contents in waters of selected areas. Extensive balance analyses of I-127 in its natural circulation (rain, surface and groundwaters) and in special isolated areas (salt formations). Ivestigations on the effects of enhanced I-127 concentrations from natural or man-made sources on the accumulation of I-129.

II. Objectives for the reporting period:

A. Investigations on the long-term translocation of I-129 in two different soil types and transfer from soil to plant.

B. "In-vivo" experiment and evaluation of the transfer of I-129 along the feed-cow-milk-pig thyroid pathway and of the transfer from feed to meat under consideration of the different animal parts.

III. Progress achieved:

A. Long-term translocation of I-129 in soils and transfer to plants

1. Methodology

The investigations on the long-term translocation of I-129 in the soil using an undisturbed soil column (a sandy soil with a high humus content) and a pasture on a river bank (an allochthone silty loam) were continued. In order to obtain representative concentration profiles in soil, 12 parallel cores uniformly positioned across the labeled areas were taken in 5 and 10-cm steps down to a depth of 100 cm and analysed for I-129 contents.

2. Results

The I-129 profiles in the soils of the monolith and the pasture exhibit clear differences in the distribution of radioiodine after comparative periods of 52 and 47 months, respectively.

The last I-129 profile taken on the pasture during 1988 confirms an unusually fast translocation of the radioiodine within the allochthone soil. About 66 % of the activity can be found in the top 10-cm soil layer. The rest of the activity is rather uniformly distributed throughout the layers between 10 and 40 cm. The soil monolith retains about 88 % of the I-129 activity in the upper layer of 10 cm after a comparable time period of 52 months.

Residence constants (in units of sec^{-1}) calculated from the time course of the radioiodine translocation in the soil monolith show values of $2.3 \cdot 10^{-9}$ and $1.0 \cdot 10^{-9}$ referring to the upper soil layers of 0-5 and 0-10 cm, respectively. Corresponding residence constants of the pasture exhibit higher values of $7.0 \cdot 10^{-9}$ and $4.1 \cdot 10^{-9}$, respectively.

Transfer factors plant/soil obtained for the monolith 39 and 52 months after contamination are $1.5 \cdot 10^{-3}$ and $2.1 \cdot 10^{-3}$ (on the basis of dry plant matter), respectively. Transfer data from the pasture 35 and 47 monthys after contamination show values of $9 \cdot 10^{-3}$ and $8.4 \cdot 10^{-3}$, respectively.

3. Discussion

The comparatively fast translocation of iodine-129 in the allochthone soil of the pasture may be explained by the fact that the ground water rises in the pasture soil in case of high water levels in the near river. In those situations anaerobic conditions are established in the soil, under which absorbed iodine may be converted into iodide, which is translocated downwards with sinking ground water levels.

The higher mobilithy of the radioiodine within the pasture soil corresponds to a higher availability of I-129 for plants and consequently to a higher I-129 uptake by plants, as found with this soil.

In order to study the long-term transfer of radioiodine under soil conditions considered typical for the pasture/land of Northern Germany, a new experimental area with a size of 200 m^2 has been selected and superficially contaminated with I-129 in July, 1988. This way, the long-term behaviour of radioiodine under soil conditions predominant in cattle breeding can be compared with that under conditions of the river bank and of the soil monolith, the latter experiencing no soil cultivation at all.

B. Transfer of I-129 along the food chain

1. Methodology

To investigate the transfer of iodine-129 on the feed-cow-milk-pig thyroid pathway under conditions of a long-term contamination, a feeding experiment with a dairy cow was performed. Pasture grass contaminated with I-129 via roots was used as labeled feed. To simulate the transfer of I-129 into human thyroid glands, 3 kg of the labeled milk collected daily were fed to a pig. The pig was used because of the physiological similarity between pigs and humans. The transfer of I-129 from feed to milk, to cow meat and to pig thyroid gland was followed for a period of 53 days. At the end of the experiment the animals were slaughtered, in order to determine the specific activity of I-129 in various butcher cuts of the cow and in the thyroid of the pig.

2. Results

The average value of the transfer factor milk/feed for I-129 resulting from the experimental period of 53 days was found to be $2.4 \cdot 10^{-3}$ $d \cdot kg^{-1}$.

Transfer factors meat/feed (in units of $d \cdot kg^{-1}$ fresh weight) obtained for 8 muscle parts ranged from $7.3 \cdot 10^{-3}$ (forerib) to $5.4 \cdot 10^{-2}$ (bestrib) with a mean value of $2.9 \cdot 10^{-2}$. Three organs (liver, kidney and thyroid) demonstrated very different transfer factors of $7.3 \cdot 10^{-3}$, $3.0 \cdot 10^{-4}$ and 4.4, respectively. The kidney exhibited a very low value possibly due to a fast metabolism occurring in this organ. The mean value of the transfer factor meat/feed of all examined tissues of the cow body (excluding thyroid) was $2.4 \cdot 10^{-2}$ $d \cdot kg^{-1}$ f.w. Transfer factors pig thyroid/milk and pig thyroid/cow fodder were 1.2 and $8.7 \cdot 10^{-3}$ $d \cdot kg^{-1}$ f.w., respectively.

3. Discussion

The mean value of the transfer factor milk/feed of $2.4 \cdot 10^{-3}$ $d \cdot kg^{-1}$ has been found to accord well with the value of $2.6 \cdot 10^{-3}$ $d \cdot kg^{-1}$ obtained for I-131 from the Chernobyl-fallout during an in vivo experiment with dairy cows undertaken in May, 1986. The results demonstrate a similar transfer feed-milk of radioiodine, whether it is taken up via root system or superficially after fallout.

The high value of the transfer factor pig thyroid/milk of 1.2 $d \cdot kg^{-1}$ f.w. supports the high radiation burden on the human thyroid when drinking I-129 contaminated milk.

IV. Objectives for the next reporting period:

Investigations on the translocation of I-129 in the soil of three experimental areas and transfer to plants.
Investigations on water samples of different origin to examine the dependency of I-129 concentrations on I-127 contents.
Inventory of I-129 and I-127 in human and animal thyroids especially with regard to areas strongly contaminated with the Chernobyl-fallout.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

D. Smidt, F.-W. Huth, A. Pfau,
Institut für Tierzucht und Tierverhalten der FAL Mariensee, FRG.

A. Georgii,
Pathalogisches Institut der Medizinischen Hochschule, Hannover, FRG.

VI. Publications:

(1) Handl, J.; Pfau, A,:
Transfer of some Chernobyl fallout nuclides in the animal product food chain.
IV. International Symposium of Radioecology on "The Impact of Nuclear Origin Accidents on Environment", Cadarache, March 14 - 18, 1988.

(2) Handl, J.; Pfau, A.:
Long-term transfer of I-129 into the food chain.
Workshop on "The Transfer of Radionuclides to Livestock" by the Commission of the European Communities, Oxford, September 5 - 8, 1988.

(3) Handl, J.; Pfau, A.; Bunnenberg, C.:
Data collection of Chernobyl-derived activity measurements and transfer studies.
Report for Associated Nuclear Services,
Epson Surrey KT 171 HA, UK, September 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-B-234-F

Institut de Biogéochimie Marine
Ecole Normale Supérieure
1, rue Maurice Arnoux
F-92120 Montrouge

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.M. Martin
Ecole Normale Supérieure
Institut de Biogéochimie Marine
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F-92120 Montrouge

Telephone number: (1) 46.57.12.86

Title of the research contract:

Artificial radionuclides transfer from the Rhone Delta to the Mediterranean

List of projects:

1. Artificial radionuclides transfer from the Rhone delta to the Mediterranean

Title of the project no.:

1. Artificial radionuclides transfer from the Rhone Delta to the Mediterranean.

Head(s) of project:

Dr. Jean-Marie MARTIN

Scientific staff:

Dr. J.M. Mouchel, Dr. P. Newton, Dr. A.J. Thomas, Mr. G. Corbierre, Mr. P. Prat.

I. Objectives of the project:

1. Relations between the deep nepheloid layer and the river input.
2. Behaviour of particles in the estuarine system:
   a. extension of the nepheloid system
   b. mixing of Rhone particles with other sources
   c. particle transit time
   d. relations between surface plume, nepheloid layer and deposited sediments.
3. Radionuclide fluxes to the Mediterranean Sea.

II. Objectives for the reporting period:

A number of preliminary evaluations were required:

- possibility of using radionuclides of well-known origin for tracing the Rhone influence off-shore;
- quantify the river particulate flux of radionuclides to the estuarine system, and compare it to atmospheric fallout when necessary;
- evaluate the constancy of the river radioactive signal with time;
- test the suitability of sampling procedures in low turbidity marine waters, and determine the concentrations of radionuclides in the 3 major sedimentary units;

- identify the area in which the Rhone radioactive fingerprint is detectable in the bottom sediments.

III. Progress achieved:

1. A general synthesis of all available data collected during various cruises from 1982 to 1986 concerning the natural and artificial radionuclides (in dissolved and particulate form) in the Rhone river and marine surface plume has been undertaken and submitted for publication (Martin § Thomas, 1988).

Radionuclides mainly originate from Marcoule reprocessing plant, with the possible exception of Co-60 also released with PWR effluents.

The average riverine flux of radionuclides including 10 gamma emitters and transuranics (Am-241, Pu-238 and Pu-239 + 240) to the Mediterranean was determined.

Comparison of riverine input with atmospheric input over the western Mediterranean basin shows that river and atmospheric inputs of Pu-239 + 240 and Cs-137 are similar or very close, whilst the river input of Pu-238 is largely predominant.

Large variations of distribution coefficients between water and particles probably indicate slow fixation kinetics as compared to the short transit time of river waters from Marcoule plant to the delta.

Yearly concentration variations are high, but suspended matter tracing may be achieved using a few more constant activity ratios, such as Pu-238/Pu-239 + 240 and area in which a measurable fraction of Rhone material may be detected in surface bottom sediments.

Concentrations of gamma emitters in phytoplankton sampled in the surface plume do not show any evidence of selective enrichment.

2. Radionuclide concentration fluctuations were investigated on a short term basis in July 1987. In the river at Arles, these variations reached one order of magnitude during the same week. They were attributed to cyclic discharges at Marcoule plant, and to a lesser extent to rapid variations of river flow. Scattered concentrations in the plume are included in this wide river concentration range; it is therefore unlikely that estuarine exchange processes may be demonstrated by such in-situ cross-sections, due to unexpectedly large short term input variations.

3. It was therefore decided that radionuclide behaviour should be further studied by in-vitro experiments. Fast distribution coefficients (FDC) were determined by a study of radioactive tracer fixation onto Rhone river and marine suspended matters, under controlled oonditions. It has been demonstrated that a considerable FDC decrease, oorresponding to a rapid mobilization of easily exchangeable Cs, should occur in seawater. On the contrary field data show the constancy of in-situ Kd's in all the surface plume, and selective chemioal extractions have shown a large predominance of a non-labile fraction. Thus, despite the likelihood of exchange processes, it is concluded that Cs isotopes associated to marine particulate material can be considered to trace Rhone particles.

4. On-going Pb-210 dating of sediment cores taken near the mouth and below the extremity of the surface plume 20 km southwards shows a rapid 50 fold decrease of sedimentation rate in the major axis of sediment transport. Clearly most of the Rhone particulate discharge remains trapped in the immediate vicinity of its mouth.

5. Activities in the deep nepheloid layer could be determined by sampling and filtration of 1200 l of waters of very low turbidity (1.6 mg/l) in order to be able to collect sufficient quantity of particulate material for analysis.On the whole they are not significantly different from those in river or plume particles, but activities of short-lived nuclides could not be accurately determined due to limitations in sample quantity.

IV. Objectives for the next reporting period:

Investigations will be focused on:

- in-vitro study of slow fixation kinetis, complementary to previous FDC determinations.
- tracing the Rhone impact on the western Mediterranean basin by determining the two-dimensional extension of Pu-238, a typical long-lived Rhone tracer, and comparing its deposition rate in sediments (core inventories) with atmospheric deposition.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None.

VI. Publications:

J.M. Martin § A.J. Thomas:
Origin, concentration and distribution of artificial radionuclides discharged by the Rhone river to the Mediterranean Sea. Submitted to J. Environ. Radioactivity, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-B-043-IRL

The University of Dublin
Trinity College
IRL- Dublin 2

Head(s) of research team(s) [name(s) and address(es)]:

Dr. I.R. McAulay
Dept. of Pure and Applied Physics
Trinity College
IRL- Dublin 2

Telephone number: 77.29.41

Title of the research contract:

Radioactivity in the sea and food in Ireland.

List of projects:

1. Reduction of radioactivity in Irish Sea subsequent to changes at Sellafield Reprocessing Plant.
2. Radioactivity in foodstuffs produced in Republic of Ireland.

Title of the project no.: 1.

Reduction of radioactivity in the Irish Sea subsequent to changes at the Sellafield Reprocessing Plant.

Head(s) of project: Dr. I.R. McAulay
Department of Physics
Trinity College,
Dublin 2, Ireland.

Scientific staff: Miss A. Hayes
Department of Physics
Trinity College,
Dublin 2, Ireland.

I. Objectives of the project:

The discharges of radioactive isotopes from Sellafield will be assessed for their effect at the Irish Coast by measurements on samples of seaweed and seawater from the Eastern coast of Ireland. The effect of new plant coming into operation at Sellafield will be determined and data will be obtained for use in appropriate models for the distribution of radionuclides in the Irish marine environment.

II. Objectives for the reporting period:

Sampling at the selected stations was to be continued. It was proposed to concentrate the sampling on the stations in the North Irish Sea to investigate as closely as possible the variation in the ratio of the caesium isotopes during the year, in addition to following the absolute 137-caesium levels. An intercalibration exercise was proposed by the United Kingdom M.A.F.F. laboratory at Lowestoft, to be organised by them. It was the intention to participate in this.

III. Progress achieved:

(1) Methodology:

Measurement of samples of fucus vesiculosus which were collected at four sampling points along the East coast of Ireland continued throughout the year. Measurement of seawater samples collected at one of the sampling stations was also carried out on a regular basis. The seaweed samples were prepared by drying and powdering. The water samples were acidified, pre-filtered and the caesium extracted by an ion-exchange resin column. Radioactivity measurements were made using a high resolution semiconductor system for both sets of samples. Forty seaweed and eight water samples were measured during the reporting period.

(2) Results:

The mean values for 137-caesium in fucus seaweed at the North Dublin station were as follows:

1984	99 Bq/kg dry weight	
1985	78 Bq/kg dry weight	
1986	45 Bq/kg dry weight	(Pre-Chernobyl)
1986	103 Bq/kg dry weight	(Post-Chernobyl)
1987	52 Bq/kg dry weight	
1988	32 Bq/kg dry weight.	

The mean values for 137-caesium in fucus seaweed at the four stations for 1988 were as follows:

Down (N. Ireland)	28 Bq/kg dry weight
North Dublin	32 Bq/kg dry weight
South Dublin	20 Bq/kg dry weight
Wicklow	16 Bq/kg dry weight.

Seawater content of 137-caesium:

1987	180 m Bq/litre
1988	86 m Bq/litre.

The main difficutly that arose during the measurement programme was due to the steep drop in 134-caesium concentrations in all samples. This has now dropped below the levels at which it can be accurately determined in seaweed. In seawater the 137-caesium to 134-caesium ratio has risen from a mean of 16 in 1987 to approximately 30 in 1988.
The intercomparison exercise organised by the M.A.F.F. laboratory at Lowestoft gave very satisfactory results. The sample was supplied as low activity labelled seawater and the mean value obtained by ten participating laboratories was 112.4 ± 10 Bq m^{-3}, and by our laboratory 112.5±5.7 Bq m^{-3}. Data obtained in this project was made available to the European Commission MARINA Committee and some has been incorporated into the database used by this Committee.

(3) Discussion:

The amount of radioactive caesium in the Irish Sea as indicated by both seaweed and seawater measurements is clearly continuing to decrease. The disturbance caused by the Chernobyl fallout has almost completely vanished though it may still be affecting the caesium isotope ratio which is sensitive to small amounts of 134-caesium in seawater.

The box model of the Irish Sea as developed in the U.K. is well supported by the results of this project, though there is some evidence that the water movement produces rather higher values than would be expected at some locations on the North-East Irish coast.

The objectives for the period have in general been achieved. The reduction in discharges reported by the Sellafield reprocessing plant have been borne out by the steep fall in concentrations of caesium at the Irish coast.

IV. Objectives for the next reporting period:

Sampling at the selected stations will be continued during 1989. In view of the difficulties in 134-caesium measurements it is proposed to use longer counting times on appropriate samples to improve the accuracy of the values determined. Investigation of a seasonal variation in 137-caesium levels will continue.

Some measurements on fish samples from inshore waters will be made during 1989. The results obtained during the course of the project will be prepared for publication.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Mr. J. Cunningham	Nuclear Energy Board, 3 Clonskeagh Square, Clonskeagh Road, Dublin 4, Ireland.
Dr. P. Mitchell	Department of Physics, University College, Dublin 4, Ireland.
Dr. N. Mitchell	Fisheries Radiobiological Laboratory, Lowestoft, Suffolk, United Kingdom.
Dr. A. Aarkrog	Risø National Laboratory, DK-4000 Roskilde, Denmark.

VI. Publications:

Title of the project no.: 2.

Radioactivity in Foodstuffs Produced in the Republic of Ireland

Head(s) of project: Dr. I.R. McAulay
Department of Physics
Trinity College
Dublin 2, Ireland

Scientific staff: Mr. D. Moran
Miss A. Hayes
Department of Physics
Trinity College
Dublin 2, Ireland

I. Objectives of the project:

To identify the radioactive isotopes present in agricultural produce in the Republic of Ireland and to assess the collective dose resulting from their consumption. To investigate the paths by which artificial radioisotopes progress through the food chain and identify the sources of the isotopes where possible. To compare the data obtained with similar studies in other member states of the European Community.

II. Objectives for the reporting period:

Further measurements to be carried out to investigate the translocation of fallout isotopes in different types of soil, especially in the poor quality soils in which high transfer to heather had been found and transfers to vegetables in such soils will be investigated. A more detailed study to take place in the area of higher than average uranium series concentrations and this to be related to domestic radon levels if possible. Collaboration with other laboratories in the European Community to continue and the relation between radioactivity levels in heather and sheep meat to be explored in association with the national laboratory of the Nuclear Energy Board. Further efforts to be made on determining population doses.

III. Progress achieved:

(1) Methodology

Detailed measurements using gamma ray spectrometry continued on soil samples taken at 5 cm depth intervals at the ten sites selected earlier in the project. Samples of tilled soil and associated food products were made where possible. Four additional sites used for upland grazing by sheep were examined in detail for transfer of caesium to heather from soil and to sheep from heather, in conjunction with the national laboratory of the Nuclear Energy Board. Transfer of caesium to fungi and tomatoes grown on peat were determined. The distribution of natural radioactivity in an area with above average uranium/radium levels was investigated in more detail; gamma ray spectrometry was also used in this study.

(2) Results

The slow downward movement of Chernobyl caesium fallout has been maintained in undisturbed soil at each of the ten sites where detailed measurements continued. Not more than 5% of the Chernobyl caesium has penetrated beyond the 10 cm level at any of the farms by May 1988 and penetration appears least on the best pastureland. Transfer to grain, vegetables and fruit is extremely low at each of the sites where produce was available, with a concentration ratio of less than 0.01 for soil to produce concentrations.

At the four upland poor quality soil sites studied for the first time in 1988, concentration ratios in excess of one were found in some cases for transfer from soil to Calluna heather. The caesium level in grazing sheep at these sites was found to have a significant correlation with levels in soil. (See attached paper which has been accepted for publication.

Measurements on milk powders produced in one area of Ireland were continued throughout the year. Levels of caesium decreased from 4-5 Bq/kg at the beginning of the reporting period to a mean of less than 2 Bq/kg at the end.

In view of the high concentration ratios found in peaty soils for heather, a series of measurements were made on commercially grown mushrooms and on the peat moss used as a growing medium. Concentration ratios of up to 0.1 were found for caesium in soil to mushroom transfer. A very limited test was carried out in which tomato plants were grown in moss peat; this experiment yielded concentration ratios for caesium from peat to tomato in the range 0.1 to 0.2.

Further measurements on a limited range of heather honey samples were carried out in 1988 and values of several hundred becquerel per kg were measured.

The area of higher than average natural radioactivity reported earlier (see attached paper which is in press) was studied in more detail. Soil levels of radium ranging up to ten times the national average have been found and these areas coincide closely with the areas in which high domestic radon levels have been found. Detailed measurements on soils from different depths to 30 cm were carried out for one site and no significant variation found in radium concentration with depth.

(3) Discussion

It has been established that very low translocation rates apply for caesium in good quality agricultural soils and also that concentration ratios to produce from such soils are small (0.01 or less). However, in peaty soils it appears that caesium is much more mobile and, in particular, concentration ratios in excess of one are found for Calluna heather growing on such soils. Very high levels of weapons test fallout caesium remain in such soils and it has been found that there is a greater mobility to vegetation for the more recently deposited caesium than for the older fraction of total caesium. In the case of peaty, acidic soils higher concentration ratios have been measured for caesium to mushrooms and to tomatoes than for produce grown on good agricultural soils.

A direction relationship has been found between caesium deposition levels in upland grazing soils and levels in sheep from those areas. The Irish Nuclear Energy Board were associated with the upland grazing sheep study and the sharing of expertise proved extremely valuable.

The identification of areas of high levels of uranium series activity in soil continued. While the principle areas identified in this way appear to match well the areas identified by Dr. Mc Laughlin of University College, Dublin, as yielding high domestic radon levels, it has not been possible to match the sampling points sufficiently precisely to give a firm relation between soil radium levels and domestic radon levels. As the current project did not initially plan to relate soil radium levels and radon levels in housing, this association will not be further investigated. However, it does appear that further work in this area could yield valuable data and the establishment of a correlation could have useful predictive properties in the context of new housing construction.

The planned objectives for the reporting period have been met in most respects. The determination of population doses has not yet been made to a useful extent due to inadequate information on the regional sources of various components of the Irish diet and on the consumption figures for some of these components. The levels of some natural radioactive isotopes in food have been very low in many cases and have proved more difficult to measure than expected; this has resulted in less data than planned being obtained during the reporting period for this part of the project.

IV. Objectives for the next reporting period:

A further and final round of sampling will be carried out at the ten selected agricultural sites to gain further information on the translocation of caesium in different types of soil.

Vegetable and other produce will be obtained in the regions of high natural radioactivity and concentration ratios established where possible.

Further efforts will be made to determine collective committed dose equivalents from dietary components obtained from different regions.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. J. McLaughlin	Department of Physics University College, Dublin, Ireland.
Dr. P. Colgan	Nuclear Energy Board 3 Clonskeagh Square, Clonskeagh Road, Dublin 4, Ireland.
Dr. R. Kirchmann	CEN-SCK, Mol, Belgium

VI. Publications:

"Radiocaesium fallout in Ireland from the Chernobyl Accident, I.R. McAulay and D. Moran. J. Radiological Protection (In press).

"Retention and transfer characteristics of radiocaesium from poor quality soils to heather and from heather to sheep". I.R. McAulay, P.A. Colgan and D. Moran. Paper presented at the Workshop on "The Transfer of Radionuclides to Livestock". Oxford, September 1988. (In press).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

C.I.E.M.A.T.
Avenida Complutense n° 22
E-28040 Madrid

Contract no.: BI6-B-195-E

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Mingot Buades
Area de Protec.Radio.y Medi.Ambiente
C.I.E.M.A.T.
Avenida Complutense n° 22
E-28040 Madrid

Telephone number: 499.01.77

Title of the research contract:

Behaviour of plutonium and americium in the marine environment.

List of projects:

1. Plutonium, Americium and stable heavy metals in marine sediment. Study of the factors governing the transport from water to the sediments.

Title of the project no.:

PLUTONIUM,AMERICIUM AND STABLE HEAVY METALS IN MARINE SEDIMENT.STUDY OF THE FACTORS GOVERNING THE TRANSPORT FROM WATER TO THE SEDIMENT.

Head(s) of project:
E.IRANZO

Scientific staff:

M.Deyá,C.Gascó,J.Guerrero,A.Jornet,E.Mingarro,P.Rivas,C.Rodriguez, L.Romero.

I. Objectives of the project:

-To study the processes controling the behaviour and distribution of radioactive and heavy metals pollutants in the marine environment of Southern coast of Spain including Palomares area.

-To determine $^{239}Pu+^{240}Pu$ and ^{241}Am inventories in the continental shelf,slope and deep ocean floor of this area.

-To examine the geochemistry of Pu,Am and heavy metals in the marine sediments and to study the processes that control the removal of Pu and Am from sea water to sediments.

II. Objectives for the reporting period:

1. Seasonal variation of suspended particles population.
2. Distribution of $^{239}Pu+^{240}Pu$, ^{241}Am, ^{137}Cs, ^{210}Pb unsupported in selected cores from slope and deep ocean floor.
3. Inventories of $^{239}Pu+^{240}Pu$, ^{137}Cs and ^{241}Am in selected cores from slope and deep ocean floor.
4. Distribution of Heavy metals fraction associated with hydrous Fe and Mn oxide coatings on the surfaces of sediment particles in selected cores of slope and deep ocean floor.
5. Chronology of sediment cores.
6. Petrography of sediments.

III. Progress achieved:

1. METHODOLOGY

In addition to the methods already specified in 1987 progress report, the procedures used for the new task are described below.

1.1 Sampling of suspended particles in sea water.

Three seasonal sampling cruises(March,July,November)were achieved.The sampling network was established after knowledge of turbidity,salinity and temperature at each station.Twelve stations were stablished with 3-4 sampling levels.Samples of suspended matter were collected using a 30 L Niskin bottles.Few hours later,water samples were filtered by a vacuum system,through preweighed 47mm Nuclepore filters-(45µm pore size)and washed repeatedly with destilled water.Filters so obtained were dried at 50°C and weighed.

1.2 Chronology of sediment cores.

Sediment cores were dated using unsupported ^{210}Pb profiles.Depending on the shape of these profiles the CIC model(constant initial concentration) or the CRS model(constant rate of supply)(1)were applied.

1.3 Petrography.

The study of petrographycal aspects has been realized by optical microscopy in the size fraction >63µm in surface sediments.

2. RESULTS AND DISCUSSION

2.1 Distribution of ^{239}Pu+^{240}Pu, ^{241}Am and ^{137}Cs concentration in sediment cores.

The distribution of plutonium,americium and cesium has been determined in selected sediment cores from continental shelf,slope and deep-sea indicated in Table 1.The anomalies found in core 31,are shown in Table 3.

Discussion

The maximum Pu,Am and Cs concentration appear within the same period of time (1960-1965 Fallout peak)according with ^{210}Pb dating of sediment in each core.

In core 31,the nearest located at southern of Almanzora river mouth,a second peak of maximum Pu and Am concentration corresponding 74-78 period has been found by analyzing intermedium slides .This anomaly could be a consequence of the washing out of Palomares area by the Almanzora river during the flood which took place in october 1973.This hypothesis has been confirmed by the higher sedimentation rate and the grain size distribution in this core slide and also by the highest Pu and Am concentration in surface sediment located between Almanzora river mouth and the core 31.Besides,the petrographycal studies at this area shows that the sediment composition is mostly terrigenous.

The average sedimentation rate decreases from shelf to deep sea,therefore the maximum Pu,Am and Cs concentration in core profiles do not appear at the same deep layers.

The depth of radionuclide penetration into the sediment generally decreases as the water depth increases.Cores in continental shelf show Pu concentration reaching 16-22 cm deep-layer and only 8-10 cm deep-layer in slope and deep sea.The Pu and Am concentration detected in layers older than 1945,could be explained by the movility of Pu in the pore water or its preferential transport by a selective mixing process.

2.2 Plutonium,Cesium and Americium inventories.

The results are shown in the Table 1.

TABLE 1 :INVENTORY(Bq/m^2)

Radionuclide		#	SHELF	#	SLOPE	#	DEEP-SEA
		34	177	08	52		
		12	128	15	<0.5	16	43
	Pu	31	529				
		30	327				
		18	254	20	78	22	18
		29	252	24	3		
		34	55	08	28		
		12	60	15	12	16	25
	Am	31	160				
		30	80				
		18	30	20	45	22	24
		29	58	24	13		
		12	442			16	
	Cs	31	676				
		30	748				
		29	512	20	390		

#Core number

Discussion

The major trend is decreasing inventories of both Pu and Am with increasing water depth.In shallow regions ,near the coast,the resuspension of fine grained material can act to enhace the scavening of particle reactive elements.This fact has been confirmed with our study of suspended particles in the area.The concentration of suspended particles at the interface water-sediment is higher in continental shelf than in deep-sea. This resuspension effect can be controlled by either biological or physical processes.Studies on benthic infauna at the area show that biogenic removal is higher in area close to the coast than in deeper sediment(2).This studies confirm the theory that biological sediment mixing processes modulate the removal of a number of elements including Pu,in coastal enviroments.In deeper waters decrease the effectiveness of boundary scavenging processes since biological removal and transport via fecal pellets production is likely o become increasingly import.

The Pu,Am inventories in the sediment cores of Continental Shelf decrease from the Almanzora river mouth to the south.This fact could confirm the indicated contribution of the river.

2.3 Heavy metals concentration

The heavy metals concentration associated with hydrous Fe and Mn oxide in selected sediment cores from slope and deep sea are:

Slope	Deep-sea
Mn: 52-260 µg/g*	Mn: 10-5500 µg/g*
Pb: 3- 20 µg/g	Pb: 4-37 µg/g
Cr: 0.9-2.1 µg/g	Cr: 0.5-1.8 µg/g
Fe: 1.4-2.4 mg/g	Fe: 1.7-3.2 mg/g

*The highest value correspond to a concrete sediment slide.

Core 16(2-3)=5500 µg/g Core 22(0-1)=1800µg/g Core 08(0-1)=260 µg/g

Discusion

In deep-sea,it has been observed higher Manganesum

concentration in surface layers than in others areas.

2.4 Chronology of sediments and sedimentation rate

To determine sedimentation rates in shelf,slope and deep-sea some cores have been selected.The estimated average sedimentation rates are the following:

TABLE 2

	Core n°	Sed.rate $g.cm^{-2}.a^{-1}$	Location
Shelf	12	0.22	North Almanzora river
	31	0.26	South Almanzora river
	29	0.29	South Almanzora river
Slope	20	0.13	South Almanzora river

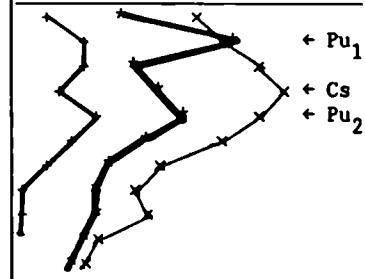
Depending on the shape of unsupported ^{210}Pb profiles obtained ,different models (CRS,CIC) were applied,using the Pu,Am and Cs profiles to check the suitability of each model.

The attention has been focused to core 31,where two peaks of maximum Pu and Am concentration but only one for Cs have been found.Not monotonous decreasing profile of unsupported lead confirmed a variation of sedimentation rate along the years,so that the C.R.S model was applied.

The results obtained are described below:

TABLA 3 : CORE 31

cm	Pb(unsup) $Bq.Kg^{-1}$	YEAR	SED.RATE $g/cm^{2}.a$	Am(+) Pu(*) Cs(×) PROFILES (Bq/Kg)
				2 4 6 8 .
1	46.1	1982	0.34	
2	45.5	1978	0.33	← Pu_1
3	55.4	1974	0.24	
4	57.3	1968	0.20	← Cs
5	41.8	1963	0.23	← Pu_2
6	36.7	1956	0.22	
7	25.3	1951	0.27	
8	23.2	1945	0.25	
9	33.8	1935	0.14	
10	12.8	1931	0.27*	
11	21.1	1921	0.15*	
..	....	....	....	



*Following sections are omitted as the uncertainty on the date results is very high.

The estimated uncertainty for the dates of the maximum peaks obtained at the Pu,Am and Cs profiles is:

Pu_1 : 1974-1980 Due to 1973 Almanzora river flood.

Cs : 1961-1971 Due to fallout

Pu_2 : 1958-1969 " " "

These results match perfectly with the hypothesis described in 2.1.

2.5 Seasonal suspended particles

The results obtained from the three samplings cruises are:

TABLE 4
Average Concentration of suspended matter (mg/L)

		Depth Level (m)	>100	500	1000	Total
SEASON	Spring	Surface	0.41	0.32	0.21	0.31
		Interm	0.39	0.39	0.14	0.31
		Bottom	0.39	0.40	0.08	0.29
	Summer	Surface	0.30	0.22	0.35	0.29
		Interm	0.27	0.23	0.26	0.25
		Bottom	0.42	0.28	0.26	0.32
	* Autumn	Surface	0.17	-	-	-
		Interm	0.40	-	-	-
		Bottom	0.26	-	-	-

*Sampling not achieved due to bad weather conditions.

Discusion

Particles population:

A decrease of population concentration has been observed with the distance to the coast in spring.In summer,the particles concentration decrease in shelf and slope,and increase in deep sea.

The higher bottom particles concentration in shelf than in deep-sea could explain a more effective scavening of radionuclides in Continental shelf.

Supporting data on temperature and salinity show that: Thermic regimen is similar to the model descript for Southern Mediterranean and the presence of Atlantic water is higher in spring and autumn than in summer.

2.6 Petrography

There are three groups of grain size sediment composition:- sand,mud and clay.The correlation of these composition parameters with depth has been studied.The linear correlation is good in the case of clays:the contents of clay increase with the depth.

A band of sand with high content of bioclastos exist along the coast at a distance of 4.5 Km and 100-200 m depth.The terrigenous sedimentation is predominant close to and south of Almanzora,Antax and Aguas rivers.

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(1) P.G.Appleby and F.Oldfield.The calculation of lead 210 assuming a constant rate of supply of unsupported ^{210}Pb to the sediment.Catena 5,1-8,1978.

(2) A.Alonso and E.López-Jamar.Infauna béntonica de las costas de Almería.Inf.Tec.Inst.Esp.Oceanogr.(1987).

IV. Objectives for the next reporting period:

-To complete analysis.
-Discussion about total results.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Miguel Deya.Instituto Oceanografico de Baleares.Muelle de Poniente,sn.Apartado 291.07080 Palma de Mallorca.

VI. Publications:

L.Romero,C.Gascó and E. Iranzo."Estudio de la distribución temporal de radionucleidos de vida larga en sedimentos marinos del S.E español".International Conference on Environmental radioactivity in the mediterranean area.Barcelona 10 a 13 de Mayo de 1988.

L.Romero,C.Gascó and E.Iranzo."Estudio de la deposicion de radionucleidos de vida larga en sedimentos marinos del Sureste Español".XXII Reunión bienal de la real sociedad española de Quimica.Murcia 26-30 de Setiembre.

L.Romero,C.Gascó and E.Iranzo."Temporal distribution of long-lived radionuclides in marine sediments at southern Coast of Spain".Rapp.Comm.int.Mer Medit,31,2(1988).Commission internationale pour L'exploration scientifique de la Mer Mediterranee.XXXI Congress.Athenes.(Grecia)

L.Romero,C.Gascó and E. Iranzo "Estudio de la deposición temporal de radionucleidos de vidad larga".Congreso de la SNE celebrado en Marbella.Octubre 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen- und Umweltforschung mbH
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BI6-B-056-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. H. Moser
Institut für Radiohydrometrie
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Telephone number: 089/318.72.561

Title of the research contract:

Investigation of the behaviour of radioiodine in aquatic and terrestrial environments under the influence of biogeochemical processes.

List of projects:

1. Investigation of the behaviour of radioiodine in aquatic and terrestrial environments under the influence of biogeochemical processes.

Title of the project no.:

Investigation of the behaviour of radioiodine in aquatic and terrestrial environments under the influence of biogeochemical processes

Head(s) of project:

Prof. Dr. H. Moser
GSF-Institut für Hydrologie
Ingolstädter Landstr. 1
D-8042 Neuherberg

Scientific staff:

H. Behrens, Dipl.-Ing.

I. Objectives of the project:

Investigation of radioiodine speciation in aquatic and terrestrial systems, esp. its conversion into organic bond under the influence of biogeochemical processes.

Study of the role of enzymatically mediated reactions in the transformation of radioiodine into organic bond. Identification of relevant enzymatic activity in water and soil. Discussion of the observed iodine conversion kinetics under the aspect of enzymatic reactions.

Study of sorption and desorption processes of radioiodine in soil/water systems by batch and column tests under the above given aspects.

Disposition of data for the description of radioiodine migration in environments under the influence of biogeochemical processes.

II. Objectives for the reporting period:

Investigation of radioiodine sorption in dynamic experiments (lab columns and outdoor facility) in dependence of:

- infiltration rate of water,
- homogeneity/inhomogeneity of soil structur,
- radioiodine sorption by natural vers. sterilized soils,
- influence of soil sample storage condition on radioiodine sorption capability.

Correlation of radioiodine conversion/sorption with enzymic activities of water/soil systems.

Modelling of iodine sorption and transformation.

III. Progress achieved:

1. Methodology

Radioiodine-sorption experiments under comparison of lab and outdoor conditions under variation of (simulated) rain intensity.

Investigation of radioiodine conversion/sorption under correlation with enzyme activities.

Development of the model presented in previous year in respect to increased iodine carrier concentration and limited turnover rates.

2. Results

Sorption of radioiodine (applied as I^-) during infiltration of spiked solutions was depending on infiltration rates: with increasing infiltration velocity the depth of radioiodine intrusion increased. Radioiodine which partly passed columnes at high flowrates turned out to be non-reacted iodide. Depth penetration of radioiodine was larger in non-homogeneous soils, however, after final sorption it was as little movable as in soils of homogeneous structure. Addition of iodine carrier decreased reaction rates and thus increased penetration of radioiodine.

Clear connection was found between enzymic activities and intensities of radioiodine conversion and sorption. FDA hydrolysis and peroxidase activity determination was applied so far. While the first is a general indicator of bioactivity, the latter may be regarded as responsible for the chemical conversions of radioiodine in surface water and soils (formation of organic iodine compounds, as well as dissolved as in the solid soil matrix). Application of further tests on bioactivity and biomass, preferably measurement of DNA is in preparation. Autoclaving proved to abolish as well iodine transformations as enzyme activities.

3. Discussion

The response of radioiodine behaviour in soil/water systems on variations of physical and chemical conditions as well as on establishment of bioactivity is in good agreement with the conjecture of beeing mediated by microbial activity. While the type of reactions is relatively uniform, consideration of all aspects with their variations in a model may become rather complex.

IV. Objectives for the next reporting period:

Continuation of the experiments in the established way under the aspect of filling gaps in order to finally be able to describe and model the behaviour of radioiodine in environments under the influence of microbial activity.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Institut de Protection et de
SÛreté Nucléaire
CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses Cedex

Contract no.: BI6-B-045-

Head(s) of research team(s) [name(s) and address(es)]:

Dr. N. Parmentier
CEA-IPSN de Fontenay-aux-Roses
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F-92265 Fontenay-aux-Roses

Telephone number: 46.54.83.35

Title of the research contract:

Consequences of sea disposal.

List of projects:

1. Consequences of sea disposal.

Title of the project no.:

CONSEQUENCES DE L'IMMERSION DE DECHETS SUR LE FOND DE L'OCEAN

Head(s) of project: N. PARMENTIER

Scientific staff: M. CHARTIER

I. Objectives of the project:

Un nouveau modèle numérique stratifié de circulation générale de l'océan a été élaboré en France. Le projet consiste en l'intercomparaison de ce modèle avec celui utilisé par les Américains sur un problème test.

II. Objectives for the reporting period:

Les données de vitesse, température et salinité provenant du modèle américain à l'état d'équilibre (après 4 000 000 de pas de temps) ont été reçues en France. Une série de simulations du même problème test avec le modèle français a permis de comparer en détail les deux modèles et de tester plusieurs méthodes numériques d'initialisation du modèle français à partir de données extérieures (ici celles provenant du modèle américain).

III. Progress achieved:

1. Rappel du contexte international et européen sur l'immersion de déchets radioactifs en océan profond

Des déchets radioactifs de faible et moyenne activité ont été immergés en Atlantique Nord-Est profond, de 1949 à 1982, par un certain nombre de pays européens. C'est essentiellement par le mécanisme de dilution par les masses d'eau océaniques que sont satisfaits les critères de sûreté de cette activité. Une analyse de sûreté de la pratique d'immersion nécessite donc la quantification exacte des processus physiques de dispersion des radioéléments dans l'océan. C'est aujourd'hui au moyen de modèles numériques sophistiqués que l'on évalue le transport et la diffusion tridimensionnelle des radioéléments dans le fluide océanique. Ces modèles sont peu nombreux dans le monde et particulièrement difficiles, voire impossibles, à valider directement par des mesures. Certains groupes d'experts internationaux (groupe SEABED de l'OCDE/AEN, groupe du Co-ordinated Research and Surveillance Programme de l'OCDE/AEN) ont ainsi recommandé que les modèles existants soient comparés entre eux afin de mieux cerner la fiabilité de leur résultats respectifs (sorte d'"intervalidation partielle relative").

Dans ce contexte, un groupe de radioprotectionnistes océanographes français a décidé de comparer le nouveau modèle de circulation générale de l'océan développé en France à un modèle américain éprouvé qui a, par le passé, été plusieurs fois utilisé dans des études de radioprotection. Le cadre scientifique de ce travail s'est inscrit dans le programme SEABED de l'OCDE/AEN. Le but était d'obtenir en Europe, et particulièrement en France, un modèle de circulation générale de l'océan capable de calculer précisément la dispersion physique des radioéléments dans l'océan, qui soit au moins d'une qualité équivalente au modèle développé Outre-Atlantique, et qui soit d'une fiabilité éprouvée. Ces objectifs ont été atteints en partie grâce à l'exercice d'intercomparaison sur un problème repère dont les résultats essentiels sont exposés ici. Le détail des résultats figurent dans le rapport [7] publié en langue anglaise.

2. Les modèles, le problème repère, la méthodologie

L'intercomparaison des modèles portent sur le modèle américain écrit par BRYAN [1], recodé par SEMTNER [2] et COX [3] et mis en oeuvre par MARIETTA [4], et le modèle français mis au point par le Laboratoire d'Océanographie Dynamique et de Climatologie et le Commissariat à l'Energie Atomique [5]. Les deux sont des modèles tridimensionnels de

circulation générale qui résolvent les équations primitives de l'océan, c'est-à-dire les équations tridimensionnelles de Navier Stokes légèrement simplifiées en supposant l'océan incompressible et hydrostatique, et en prenant en compte l'hypothèse de Boussinesq et du toit rigide. Les méthodes aux différences finies sont utilisées pour les deux modèles mais avec des schémas notablement différents : grille d'ARAKAWA [6] type B pour le modèle américain et type C pour le modèle français ; conservation de l'énergie au cours du mouvement des masses d'eau pour le modèle américain et conservation de l'enstrophie potentielle pour le modèle français, coordonnées sphériques pour le modèle américain et coordonnées curvilignes pour le modèle français. Les modèles diffèrent aussi par d'autres points moins importants (filtres temporels, terme de dissipation de la quantité de mouvement, méthode de surrelaxation Rouge-Noir pour la résolution de l'équation elliptique de la fonction de courant dans le modèle français,...). Les deux modèles fournissent les vecteurs vitesse tridimensionnels aux noeuds d'un maillage lui-même tridimensionnel. Ils calculent aussi la diffusion d'un traceur quelconque aux noeuds du maillage.

Le problème repère a été élaboré par le groupe d'Océanographie Physique du programme SEABED de l'AEN [4]. C'est un océan rectangulaire à fond plat qui représente très schématiquement l'Océan Atlantique Nord. Il est excité par une tension de vent zonale et des flux de surface de chaleur et de salinité qui représentent approximativement le vent et les flux moyens en Atlantique Nord. Bien qu'académique et très simple, ce problème repère reprend en fait les aspects saillants de la dynamique océanique de l'Atlantique Nord, ce qui le rend particulièrement adapté à cet exercice d'intercomparaison de modèles de circulation générale. Il est important de noter que le pas de grille horizontal a été fixé à 2° de latitude et de longitude, et la résolution verticale à 6 niveaux : la résolution spatiale est donc pauvre, mais permet de réduire le nombre de noeuds du maillage et donc le coût de l'intercomparaison.

De même, la méthodologie suivie a été dictée par les contraintes de coût de calcul. L'idée fondamentale est que les besoins de la radioprotection justifient la simulation d'une circulation générale à l'équilibre. Cet état n'existe bien sûr pas dans la réalité, mais on peut considérer que cet état d'équilibre est proche (en première approximation) de la moyenne annuelle de la circulation générale. On considère notamment que les variations saisonnières et interannuelles sont d'une moindre importance pour les calculs de protection radiologique concernant les océans profonds. Il semble donc justifié de comparer les modèles sur un problème de circulation à l'équilibre. Mais atteindre un état d'équilibre à partir de l'état de repos exige des temps d'intégration du modèle extrêmement longs. Il a donc été décidé

d'adapter une méthodologie qui permettre de conclure à moindre frais de calcul. Ainsi seul le modèle américain a-t-il été intégré de l'état de repos jusqu'à l'équilibre, qui a été atteint après 4 000 000 de pas de temps.

Le modèle français a été intégré à partir des champs de température et de salinité (c'est-à-dire de densité) obtenus à l'équilibre avec le modèle américain. Les mêmes tensions de vent et flux de surface sont imposés au modèle français. Après intégration, le modèle français doit conduire à un état d'équilibre plus rapidement qu'à partir de l'état de repos. On suppose même que la période d'intégration sera courte parce que les deux états d'équilibre ne seront pas très éloignés.

Plusieurs méthodes de reprise de simulation à partir des champs de densité donnés par le modèle américain ont été testées : méthode diagnostique, méthode pronostique, méthode d'accélération de convergence vers l'état d'équilibre et méthode semi-diagnostique robuste. Parallèlement aux résultats sur l'intercomparaison proprement dite, nous avons obtenu certaines informations sur la validité de ces méthodes pour obtenir un état d'équilibre à partir de champs de densité connus. L'idée qui sous tend ces tests est de cerner les méthodes susceptibles de partir de champs de densité donné par des mesures in-situ et d'intégrer le modèle pour obtenir la circulation générale. Ceci permettrait de combiner les acquis des mesures avec les avantages du modèle.

Nous ne donnerons ici que les résultats principaux concernant l'intercomparaison proprement dite. Les résultats concernant ces méthodes sont ici brièvement exposés dans la conclusion ; les résultats détaillés font l'objet d'un rapport en langue anglaise actuellement en préparation [7].

La méthode diagnostique consiste à imposer un champ de densité (c'est-à-dire de température et de salinité) constant dans le temps, et à ne calculer donc que la vitesse. La convergence vers l'état d'équilibre est rapide, de l'ordre de quelques semaines de simulation. Dans la méthode pronostique au contraire, les champs de vitesse et les champs de température et salinité sont variables. Seul l'état initial de température et salinité est prescrit : c'est la méthode qui se rapproche le plus de la réalité. La convergence vers l'état d'équilibre peut malheureusement être très lente si l'état initial est éloigné de l'état d'équilibre, comme cela a été demontré avec le modèle américain sur le problème repère (état d'équilibre obtenu en 4 000 000 de pas de temps). La méthode d'accélération de convergence vers l'état d'équilibre [8] permet de remédier en partie à cet inconvénient. Cette méthode consiste à distordre la physique du modèle pour accélérer les phénomènes

abyssaux trop lents et ralentir les ondes de gravité trop rapides en comprimant la bande de fréquence du modèle. Ceci est obtenu en étirant le temps d'un facteur α :

$$t' = \frac{t}{\alpha}$$

et en distordant la stratification en densité d'un facteur γ :

$$N'^2 = N^2 \frac{\gamma}{\alpha} \quad (N^2 = \text{fréquence de Brunt-Väisälä})$$

Le modèle à physique distordue doit converger vers le même état d'équilibre que le modèle à physique sans distorsion car les distorsions ne portent que sur les dérivées temporelles. La méthode semi-diagnostique robuste [9] est une extension de la méthode diagnostique pour prendre en compte les calculs pronostiques. Elle consiste à introduire dans l'équation de conservation de la température et de la salinité des termes fictifs de rappel vers une température et une salinité données. La constante de rappel est choisie variable avec la profondeur : elle est prise grande dans les couches de surface (calcul très diagnostique) et faible dans les couches du froid (calcul très pronostique) afin que le champ de masse s'ajuste au fond aux contraintes de la dynamique du modèle. Cette méthode combine les avantages de la méthode diagnostique avec une meilleure prise en compte de la dynamique théorique simulée par le modèle (moins grande sensibilité des résultats de simulation aux erreurs de mesure contaminant les données d'entrée).

3. Résultats

Les champs de vitesse obtenus pronostiquement avec le modèle américain et diagnostiquement avec le modèle français (avec les mêmes champs de température et de salinité) sont en bon accord (voir figures 1). Le schéma de circulation est très semblable, et le maximum des vitesses à chaque niveau est pratiquement égal, excepté à la profondeur 1150 m où la circulation est lente et plutôt cyclonique dans le modèle américain et nettement anticyclonique dans le modèle français. Ce léger écart pourrait résulter de différences dans les algorithmes d'estimation de la pression hydrostatique intégrée, qui conduiraient à des niveaux de "non-mouvement" qui ne coïncident pas exactement.

Un bruit d'origine numérique apparaît clairement sur les résultats du modèle français à haute latitude. Ce bruit se signale par sa structure géométrique en bande qui montre que la cause en est l'emploi de la grille C [10]. Il est maintenant connu [5] que la grille B (utilisé

dans le modèle américain) est meilleure que la grille C quand est faible la résolution spatiale (définie comme le rapport du rayon interne de Rossby sur le pas de grille). A haute latitude dans le problème test, le pas de grille horizontal de 2° conduit à une résolution faible qui défavorise le modèle français. Nous avons par ailleurs prouvé cette conclusion en effectuant une simulation du problème test avec une résolution double (pas de grille de 1°) avec le modèle français : le bruit numérique disparaît alors totalement.

Des simulations pronostiques ont été effectuées avec le modèle français à partir des champs initiaux de densité provenant du modèle américain, avec les mêmes excitations par tension de vent et flux de chaleur et salinité. Les figures 2 montrent le résultat après 24 000 pas de temps (soit 500 jours). La circulation et les champs de température et salinité ont évolué, mais en gardant une structure assez proche de celle des résultats américains. Quelques différences significatives sont apparues néanmoins : la vitesse dans les couches de surface est plus grande dans les résultats américains, mais la vitesse dans les couches profondes est plus grande dans les résultats français. A 1150 m, comme dans les simulations diagnostiques, la circulation est cyclonique dans le modèle américain et anticyclonique dans le modèle français. On note enfin dans le modèle français un bruit d'origine numérique aux hautes latitudes, qui est dû à l'emploi de la grille C avec une résolution pauvre.

4. Conclusions

Cette étude comparative du modèle français de circulation générale de l'océan avec un modèle équivalent américain, sur un même problème repère, a conduit aux conclusions principales suivantes :

i - Les résultats comparés des simulations avec les deux modèles, en bon accord qualitatif et quantitatif, montrent que ces modèles simulent les mêmes aspects dynamiques et thermodynamiques de la circulation générale.

ii - Des differences significatives existent néanmoins entre les résultats, qui prouvent que les techniques numériques de résolution des équations ont une influence non négligeable sur la circulation générale simulée par ces modèles. Toutefois, cette étude a montré que ces différences ne semblent pas de nature à provoquer des divergences considérables dans la simulation de la dispersion des radioéléments : ces modèles paraissent donc adapté aux exigences de radioprotection.

iii - Les modèles ayant été élaborés et utilisés indépendamment, on peut conclure après cette étude que la probabilité est très faible qu'ils contiennent encore de graves erreurs de programmation.

iv - Le modèle français, très récent, peut maintenant être considéré comme "partiellement validé" relativement au modèle américain plus ancien.

v - L'utilisation optimale du modèle français exige une résolution spatiale suffisamment fine pour éviter les bruits d'origine numérique dans les résultats.

Ces conclusions répondent aux questions des groupes d'experts concernant la fiabilité des modèles de circulation générale de l'océan pour les problèmes de radioprotection. Au vu de ces résultats positifs, un groupe français s'est constitué pour mettre en oeuvre le modèle français, de fiabilité maintenant éprouvée, pour la simulation de la circulation générale tridimensionnelle de l'Océan Mondial.

Cette étude a permis de plus de conduire à des conclusions scientifiques supplémentaires concernant certaines méthodes de simulation d'une circulation de l'océan en équilibre :

1°) La méthode diagnostique permet, à partir de champs de température, de salinité et de tension de vent, d'obtenir avec une bonne précision la circulation de l'océan. Cette méthode est néanmoins sensible au bruit numérique dû à l'emploi de la grille C, même quand les champs de température, de salinité et de vent sont bien lisses.

2°) La méthode d'accélération de convergence vers l'état d'équilibre apparaît stable quand elle est appliquée au modèle français. De plus, une simulation effectuée avec la méthode d'accélération de convergence (pas de temps de 1/2 journée) a abouti au même état de quasi équilibre qu'une simulation beaucoup plus coûteuse (pas de temps d'1/2 heure) effectuée avec la méthode pronostique : les différences sont inférieures à un pour-cent. La méthode d'accélération de convergence paraît donc particulièrement utile pour obtenir à moindre frais la circulation générale de l'océan.

3°) La méthode semi-diagnostique robuste a montré qu'elle combinait les avantages des méthodes diagnostique et pronostique. Mais cette méthode reste encore sensible aux bruits d'origine numérique dus à la grille C.

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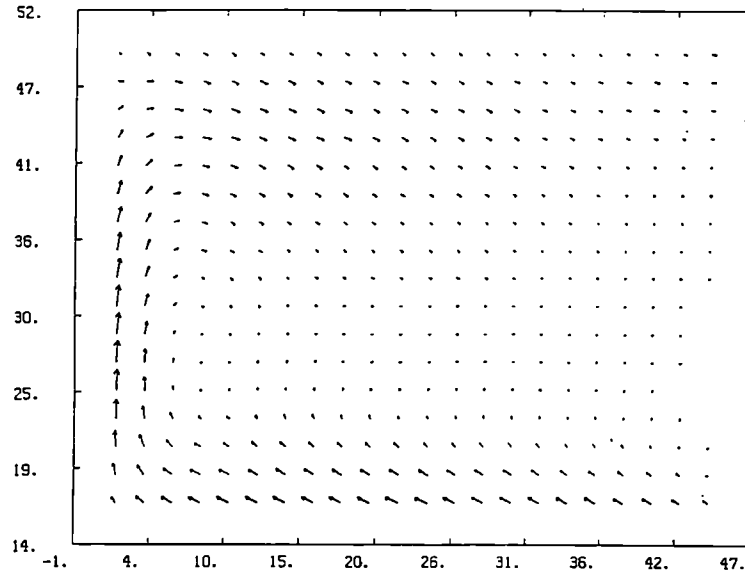
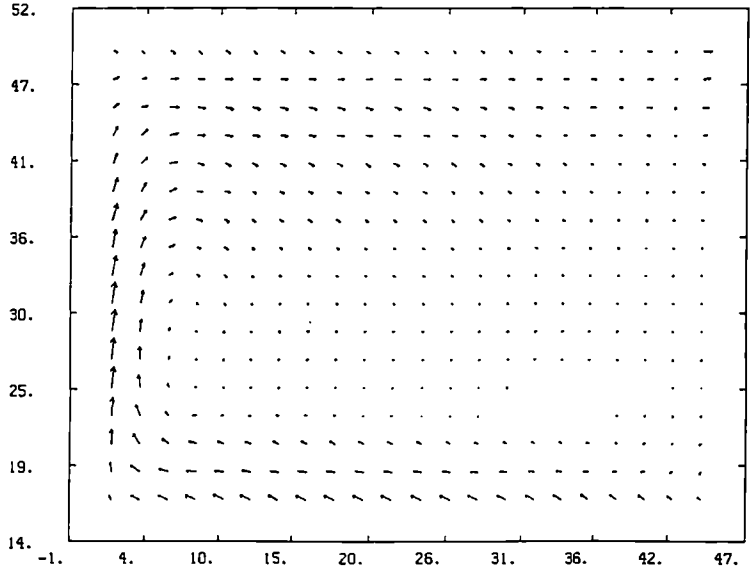
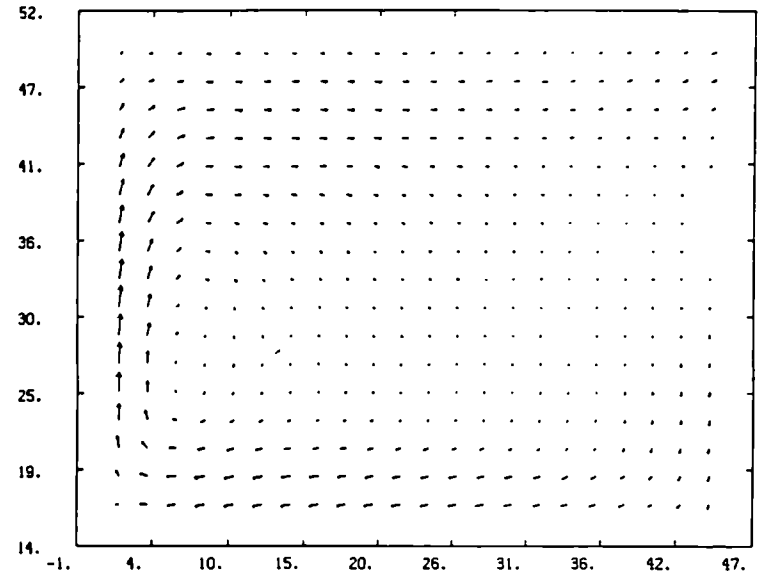


modèle américain

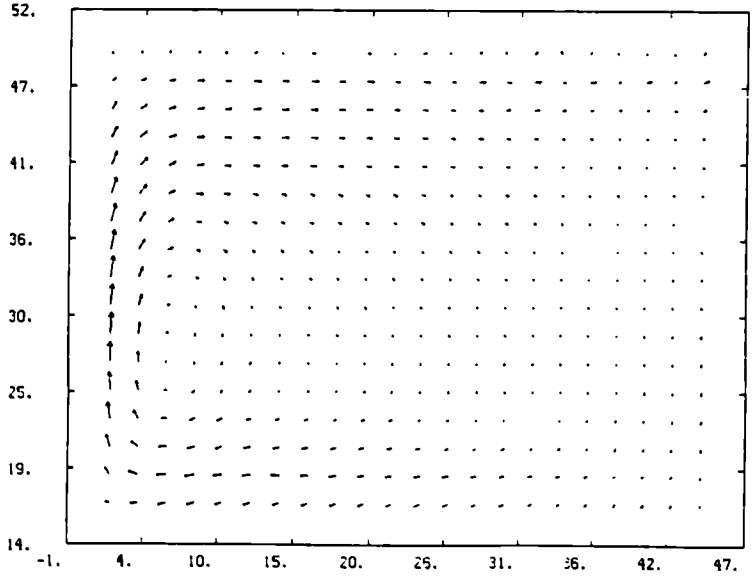


modèle français

Figures 1 : vitesse horizontale à 25m.

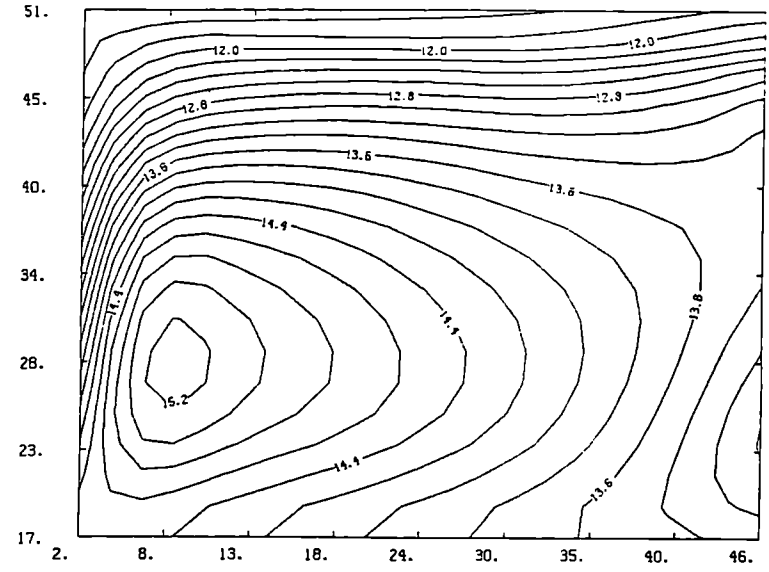


modèle américain

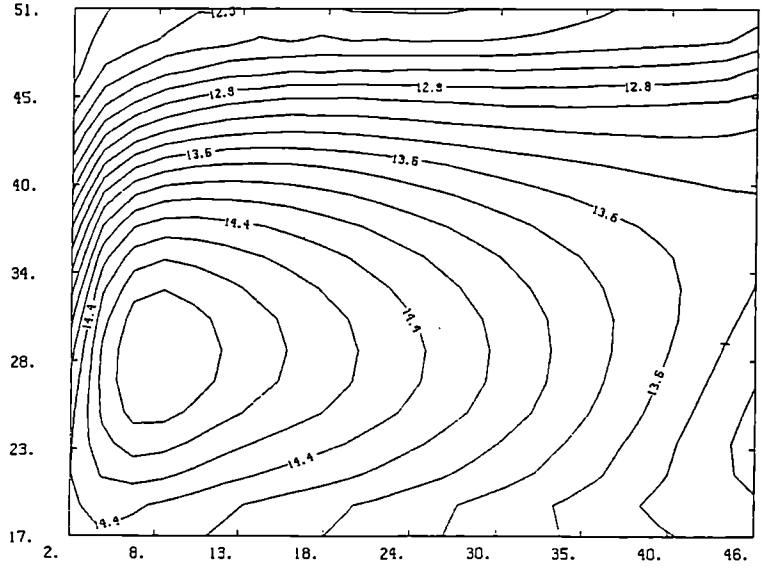


modèle français

Figures 2 : vitesse horizontale à 150m.



modèle américain



modèle français

Figures 2 (suite) : température à 500m (intervalle des contours 0.2°C)

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

M. MARIETTA - Sandia National Laboratories
ALBUQUERQUE
New-Mexico 87 185

(ETATS UNIS D'AMERIQUE)

V. Publications:

CHARTIER M. "Comparison of three-dimensional ocean general circulation models on a benchmark problem" (en préparation).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-B-200-UK

Ministry of Agriculture,
Fisheries and Food
Directorate of Fisheries Research
Fisheries Laboratory
GB- Lowestoft, Suffolk NR33 OHT

Head(s) of research team(s) [name(s) and address(es)]:

Mr. R.J. Pentreath
Fisheries Laboratory
Ministry of Agriculture,
Fisheries and Food
GB- Lowestoft, Suffolk NR33 OHT

Telephone number: (0502)562244

Title of the research contract:

Studies of the geochemical behaviour of artificial and natural radionuclides in coastal waters.

List of projects:

1. Studies of the geochemical behaviour of artificial and natural radionuclides in coastal waters.

Title of the project no.:

1. Studies of the geochemical behaviour of artificial and natural radionuclides in coastal waters.

Head(s) of project:

Dr D S Woodhead

Scientific staff:

Dr P J Kershaw, Dr D J Swift, Dr S J Malcolm
Mr B R Harvey, Mr M B Lovett, Mrs S J Boggis

I. Objectives of the project:

The main objective of the project is to obtain a detailed understanding of the interactions of a variety of radionuclides with suspended and settled sediments in coastal waters so that the long-term behaviour and distribution of certain long-lived radionuclides can be predicted under varying environmental conditions. The detailed behaviour and distributions of natural and artificial radionuclides in marine sediments are being determined and the chemical, biological and physical processes controlling the distributions are being investigated.

II. Objectives for the reporting period:

(i) The analysis and interpretation of past data on the distributions of long-lived radionuclides in the sediments of the north-east Irish Sea will be completed.

(ii) A major research vessel cruise is scheduled for November 1988 to resurvey the distributions of long-lived radionuclides in the waters and sediments of the Irish Sea.

(iii) The laboratory experiments with fluidized beds will be extended to examine the behaviour of plutonium using ^{237}Pu as a tracer.

(iv) The development and validation of the Irish Sea multi-box model will continue.

III. Progress achieved:

(i) The radiochemical analyses of the water and sediment samples collected from the Irish Sea prior to 1988 have been completed. All the data have been entered into computer files in preparation for analysis and interpretation. These are very substantial data sets and staff changes during the year have delayed the intended progress towards publication.

(ii) A 21-day research vessel cruise to the Irish Sea was completed in November 1988. All the main objectives of the cruise were achieved including:

(a) Sediment cores were collected at 73 stations in the Irish Sea and sub-sampled for gamma-spectrometric and radiochemical analyses to determine the inventories of the fission-product and transuranic nuclides in the seabed. Limited information will also be gained of the nuclide depth distributions within the seabed at the majority of stations.

(b) Surface and bottom water samples were obtained at 7 stations and surface water alone at 28 stations (the water column in the Irish Sea is expected to be well-mixed) for the analysis of Cs radionuclides, and for the transuranic radionuclides in both filtrate (< 0.45 μm) and the particulate fractions. For the transuranic nuclides, the samples were partially processed on board, including, at 14 stations, a determination of chemical speciation (oxidation state).

(c) Equipment was deployed on the seabed WSW of St Bee's Head in the north-east Irish Sea to record the near-bottom current velocity profile and suspended load as a function of time. Complementary samples of water, suspended load and seabed sediment from the ship, at anchor nearby, to determine the rate of scavenging of ^{234}Th from the water column as a function of the phase of the tidal cycle; two sets of samples were collected over periods of 25 h and 10 h.

(d) Investigations were made of the chemistry of sediment interstitial waters at 7 stations. Eh and pH were made in the sediment cores and reducing conditions were found below a few cm from the sediment surface. Concentration profiles of Fe(2+) and Mn(2+) in pore waters processed under nitrogen were determined at 1 cm resolution and confirmed the general pattern found in earlier studies. Consistently higher concentrations of these cations were found in association with the muddier (finer grained) sediments. The profiles also showed some variation with site and, in particular, the influence of animal burrows.

Ra-226 concentrations were also measured in the pore waters and a two-fold increase was found from the sediment surface to a depth of 25 cm.

(iii) The laboratory experiments with fluidized bed reactors have examined the effects of changing chemical conditions, simulating processes observed to occur in Irish Sea sediments, on the partition and adsorption of Cs-134 and Pu-237 added at tracers. Processes which affect the nature of the solid surface of the sediment, e.g. Fe and Mn reduction, have an apparent effect on the partition of Cs-134 but not on that of Pu-237.

(iv) Environmental observations of the Th-234/U-238 disequilibria in filtered seawater, suspended particulates and surface seabed sediments have been used to investigate the rate of scavenging of particle reactive contaminants from the water column. The parameter values obtained concerning this process have been incorporated into the Irish Sea model (MIRMAID) to improve the description of the behaviour of plutonium. These developments have improved the fit between the observed and predicted distributions of plutonium, although the nuclide concentrations remain too high near the source and too low in more distant regions.

IV. Objectives for the next reporting period:

(i) Complete the radiometric analysis of the water and sediment samples collected on the November 1988 research vessel cruise.

(ii) Continue with the interpretation and writing up of the pre-1988 data on radionuclide concentrations in seawater and sediment.

(iii) The investigation of nuclide scavenging by particulates resuspended by tidal currents, using natural radionuclide tracers, will continue. The available time-series data investigating seasonality of the scavenging process will be written up.

(iv) The results of the interstitial pore water studies will be written up.

(v) The development and validation of the Irish Sea model (MIRMAID) will be continued.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None

VI. Publications:

Kershaw, P. J., Gurbutt, P. A., Young, A. K. The use of Th-234/U-238 data to control scavenging in a water quality model. ICES C.M. 1988/C:5 (13 pp. in mimeo).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université de Nantes
Laboratoire de Biochimie
et Radiobiochimie
Chemin de la Houssinière, 2
F-44072 Nantes Cedex

Contract no.: BI6-B-047-F

Head(s) of research team(s) [name(s) and address(es)]:

Prof. J. Pieri
Lab. de Biochimie et Radiobiochimie
Université de Nantes
Chemin de la Houssinière 2
F-44072 Nantes Cedex

Telephone number: 40/74.00.26

Title of the research contract:

Ligands of technetium and transfer.

List of projects:

1. Ligands of technetium and transfer.

Title of the project no.:

Subcellular ligands of technetium in living organisms.

Head(s) of project:

Prof. J. PIERI

Laboratoire de Biochimie et Radiobiochimie

Rue de la Houssinière 2 - UNIVERSITE DE NANTES

F- 44072 NANTES Cedex 03

Scientific staff:

Pr. J. PIERI, J. GALEY, F. GOUDARD.

I. Objectives of the project:

Etude des ligands bioorganiques du Tc cytosolique

Etude des métabolites liant le Tc

Affinité du Tc selon le ligand (sites de fixation)

II. Objectives for the reporting period:

Etudier le devenir des différents ligands bioorganiques du technetium au niveau subcellulaire. Les organites les plus importants de la détoxification se trouvent être les lysosomes. Au niveau soluble, la recherche de protéines spécifiques est poursuivie ainsi que l'étude de leur liaison avec le technetium en relation avec la présence de métaux comme le cuivre, le zinc, le cadmium et le fer.

III. Progress achieved:

Transfert aux biota au niveau moléculaire.

Sur des homards contaminés au laboratoire de Radioécologie Marine de La Hague par une méthode définie précédement, nous avons étudié la répartition du technetium après séparation du culot 12 000 g (14,4 % de la radioactivité globale) sur gradient de métrizamide . Les courbes de phosphatase acide et de cytochrome oxydase localisant les lysosomes et les mitoohondries témoignent de la bonne séparation des organites cellulaires. Le ^{95m}Tc mesuré essentiellement dans les fractions de 1 à 7 ($\simeq$ 72 %) de densité moyenne 1,138 g/cm3 est dono associé aux lysosomes.

La loealisation du teohnétium est essentiellement cytosolique ($\simeq$ 76, 5 %) . l'analyse plus détaillée du fractionnement des constituants cytosoliques en filtration sur gel a montré que le radionucléide ne se fixerait pas sur l'hémocyanine synthétisée dans cette glande digestive, ni sur la ferritine qui est un des ligands de l'américium. Les ligands du technétium se retrouvent à 12 000 D coélués avec du cuivre (59 %) et du zinc. Ils comprennent trois fractions : l'une oationique (I) et deux autres anioniques (III et IV) qui ont la caractéristique de présenter un fort pourcentage en histidine. Or les ligands imidazole ou carboxyliques sont des ligands majoritaires (analyse d'acides aminés) susceptibles de fournir des liens avec le teohnétium à l'état réduit probablement formé par les mécanismee d' oxydo-réduotion oellulaires. Chaque fraction technétiée est liée au cuivre (environ 4 atomes de cuivre par molécule pour les fractions III et IV); mais, la majorité du ouivre est éluée indépendamment du technétium (fraction II). Or, les complexes imidazoles produisent des ohamps forts oréant des liens covalents stables. Si de tels liens existent avec le technétium, lui aussi métal de transition du groupe d , il est difficile de prévoir une élimination rapide du radionucléide stocké à ce niveau, sinon par le turn-over protéique.

Transfert aux sédiments estuariens

Indépendamment du ^{137}Cs, nous avons donné des résultats en spectrométrie γ sur le ^{228}Ra, ^{214}Bi, ^{40}K, ^{134}Cs dans l'estuaire de la

Loire au niveau des sédiments côtiers sur les mêmes sites de prélèvement que le ^{137}Cs.

Les teneurs en radioisotopes sont réglées essentiellement par 2 paramètres : la teneur en ligands argileux (concentrations les plus élevées) et la proximité du chenal. Il existe une corrélation très nette entre le ^{228}Ra et le ^{214}Bi ($[^{228}Ra] = 1,76 [^{214}Bi]$). Le ^{40}K peut être attribué à la présence dans les fractions sableuses de matériaux provenant du démantèlement de l'encaissant géologique tout proche. Les teneurs en ^{137}Cs sont nettement plus faibles que celles trouvées en aval des centrales nucléaires en fonctionnement sur le cours de la Loire. Les échantillons sableux sont les moins riches en radioisotopes, en raison de leur faiblesse en éléments argileux et de l'origine non ligérienne de ces sables.

En coopération avec le Professeur A. Cremers (Leuven) , une caractéristique de la liaison du ^{137}Cs dans les sédiments côtiers a pu être dérivée des résultats d'adsorption. Les valeurs de K_D ont pu être interprétées sur la base des propriétés du sédiment (nombre de sites spécifiques), de la sélectivité des ions et de la composition de l'eau de mer.

IV. Objectives for the next reporting period:

- Transfert du ^{137}Cs aux biota (niveau moléculaire) en milieu aquatique (Loire) à partir de sédiments estuariens (basse activité). L'interprétation quantitative du radiocésium sur les sédiments sera faite selon la technique du masquage par AgTu pour quantifier les sites spécifiques telle qu'elle a été définie par le Professeur A. Cremers et ses collaborateurs.
- Transfert aux biota (milieu aquatique) des produits du Radon : Polonium et Plomb.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Laboratorium voor colloidale scheikunde Katholieke Universiteit te Leuven

Laboratoire de Radioécologie marine CEA. La Hague.

VI. Publications:

- J. GALEY, F. GOUDARD, J. PIERI, S.W. FOWLER and F.P. CARVALHO Tissue and subcellular distribution of ^{252}Cf and ^{241}Am in the seastar Marthasterias glacialis. Marine Biology 75, 253-259 (1983)
- F. GOUDARD, J. GALEY, J. PIERI, S.W. FOWLER, S. HEUSSNER and J. LA ROSA. Intracellular localization and binding of technetium-95m in the seastar Marthasterias glacialis. Marine Biology 85 , 43-50 (1985).
- J. GALEY, F. GOUDARD, J. PIERI, P. GERMAIN and S.G. GEORGE. ^{241}Am binding-components in the digestive gland cells of the marine prosobranch Littorina littorea . Comp. Biochem. Physiol. 85 A 333-340 (1986).
- F. GOUDARD, J. GALEY, J. PIERI, M. MASSON and S.G. GEORGE. Localization of ^{95m}To and ^{241}Am subcellular binding ligands in the lobster (Homarus gammarus) in relation to some stable metals (Cu, Fe, Zn and Cd).

Soumise à Comparative Biochemistry and Physiology.

A. CREMERS, J. VANCLUYSEN, A. ELSEN, A. MAES, J. PIERI and J. NIKODIC. Radiocaesium interception in estuarine sediments : A quantitative interpretation.
Soumise à Nature.

J. NIKODIC and J. PIERI. Sorption kinetics of 60-Co by main clay-type minerals present in estuaries concentration, pH, salinity and temperature effects on K_D values equilibrium dialysis application. In : Application of distribution coefficients to radiological assessment models. C.E.C. Elsevier applied science publishers (1985).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon, OX11 ORQ

Contract no.: BI6-B-048-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Stather
Biomedical Effects Department
NRPB
Chilton, Didcot
GB- Oxon OX 11 ORQ

Miss F.A. Fry
Environmental Measurements Dept
NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: 0235/83.16.00

Title of the research contract:

Behaviour of radionuclides in the environment.

List of projects:

1. Soil-to-plant transfer factors for radionuclides.
2. The speciation of radionuclides in plants and foodstuffs and the influence of this on their gastrointestinal uptake.

Title of the project no.: 1

Soil-to-plant transfer factors for radionuclides

Head(s) of project: Dr D S Popplewell

Scientific staff: Dr A F Nisbet

I. Objectives of the project:

To investigate the dynamics and time dependent transfer of ^{239}Pu, ^{241}Am, ^{90}Sr and ^{137}Cs into food crops from soils of widely differing textural classes, representative of those found in the EEC.

II. Objectives for the reporting period:

(i) To continue the investigation into the effect of glyphosate (the active ingredient in the widely used herbicide 'Tumbleweed'/ 'Round Up') on the uptake of radionuclides to food crops.

(ii) To continue the investigation into the time dependent transfer of radionuclides to food crops from soils radio-labelled in 1984.

(iii) To determine radionuclide concentrations in soil water under field and greenhouse lysimeter conditions to assess more accurately radionuclide availability to crops.

III. Progress achieved:

Methodology

(i) Peas and carrots grown during 1987 in radio-labelled greenhouse tubs with and without soil applications of glyphosate have undergone radiochemical analysis. A lettuce crop has subsequently been harvested from these same tubs during 1988 and awaits analysis.

(ii) In October 1988 spring cabbage was sown in the lysimeters containing previously radio-labelled peat, sand and loam. Winter barley harvested in August 1988 from these same lysimeters is currently undergoing radiochemical analysis. Concentration ratios for these cabbage and barley crops will be compared to those obtained in 1985 and 1986 harvests respectively.

(iii) Soil water has been routinely collected from the greenhouse lysimeter soils and from reclaimed land and uplands in north west England throughout 1988, by porous ceramic cups. To complement this work a second 'destructive' method using immiscible liquid-high speed centrifugation has been employed to extract the interstitial water from soil cores brought back to the laboratory. All samples undergo radiochemical analysis. Uptake will be related to the activity concentration in soil solution, weighted according to the growth rate of the crop. These data will permit a better understanding of the dynamics of the uptake of radionuclides by crops from soil solution.

Results

(i) Soil-to-plant concentration ratios for peas and carrots have been calculated for ^{239}Pu, ^{241}Am, ^{90}Sr and ^{137}Cs. Data for ^{241}Am are presented below.

Soil type* and treatment	Activity concentration, Bq kg^{-1} dry mass		Concentration ratio
	Soil	Peas	
Clay +	2010 ± 58	0.109 ± 0.011	$5.43 \pm 0.59 \times 10^{-5}$
Clay -	2110 ± 114	0.142 ± 0.012	$6.74 \pm 0.69 \times 10^{-5}$
Loam +	1400 ± 58	0.074 ± 0.011	$5.29 \pm 0.82 \times 10^{-5}$
Loam -	1520 ± 122	0.027 ± 0.008	$1.77 \pm 0.54 \times 10^{-5}$
Peat +	4570 ± 232	0.076 ± 0.009	$1.65 \pm 0.21 \times 10^{-5}$
Peat -	4020 ± 157	0.065 ± 0.009	$1.62 \pm 0.23 \times 10^{-5}$
Sand +	1380 ± 52	0.270 ± 0.050	$1.96 \pm 0.37 \times 10^{-4}$
Sand -	1290 ± 31	0.224 ± 0.030	$1.89 \pm 0.26 \times 10^{-4}$
	Soil	Carrots	
Clay +	1997 ± 80	0.599 ± 0.038	$3.07 \pm 0.23 \times 10^{-4}$
Clay -	2149 ± 75	0.510 ± 0.023	$2.40 \pm 0.14 \times 10^{-4}$
Loam +	1097 ± 32	0.166 ± 0.016	$1.56 \pm 0.16 \times 10^{-4}$
Loam -	1166 ± 37	0.148 ± 0.012	$1.28 \pm 0.11 \times 10^{-4}$
Peat +	4155 ± 94	0.122 ± 0.012	$2.93 \pm 0.30 \times 10^{-5}$
Peat -	3722 ± 90	0.099 ± 0.012	$2.64 \pm 0.33 \times 10^{-5}$
Sand +	1263 ± 28	0.284 ± 0.017	$2.25 \pm 0.14 \times 10^{-4}$
Sand -	1408 ± 30	0.369 ± 0.024	$2.62 \pm 0.18 \times 10^{-4}$

	Soil	Peel	
Clay +	1997 ± 80	6.502 ± 0.245	$3.60 \pm 0.20 \times 10^{-3}$
Clay -	2149 ± 75	8.957 ± 0.535	$4.17 \pm 0.29 \times 10^{-3}$
Loam +	1097 ± 32	10.533 ± 0.612	$9.60 \pm 0.63 \times 10^{-3}$
Loam -	1166 ± 37	5.864 ± 0.250	$5.03 \pm 0.27 \times 10^{-3}$
Peat +	4155 ± 94	2.697 ± 0.113	$6.49 \pm 0.31 \times 10^{-4}$
Peat -	3722 ± 90	3.331 ± 0.164	$8.95 \pm 0.49 \times 10^{-4}$
Sand +	1263 ± 28	10.460 ± 0.490	$8.28 \pm 0.43 \times 10^{-3}$
Sand -	1408 ± 30	11.604 ± 0.457	$8.24 \pm 0.37 \times 10^{-3}$

* Note: '+' = plus Glyphosate;
'-' = without Glyphosate.

(ii) Crops to be harvested Spring 1989. Results awaited.

(iii) A preliminary comparison of data using destructive and non destructive methodologies for obtaining soil solution are given below. These soil water data are from land that has been reclaimed from the sea in north west England.

Depth (date of sample) mm	Concentration in soilwater, mBq l^{-1} Centrifuge Method			
	^{137}Cs	^{239}Pu	^{241}Am	pH
0 - 50 (Nov 87)	< 200	14	15	7.5
0 - 50 (Nov 88)	< 200	7	12	7.8
50 - 100 (Nov 87)	< 200	11	15	8.3
50 - 100 (Nov 88)	< 200	3	14	8.0

Date of sample	Concentration in soilwater, mBq l^{-1} Porous Cup Method (100 mm)			
	^{137}Cs	^{239}Pu	^{241}Am	pH
Jan 88	< 200	1.2	2.0	7.4
Feb 88	< 200	0.7	1.9	7.3
Mar 88	< 200	0.5	2.1	7.0
Apr 88	< 200	0.5	1.3	6.9
Jun 88	< 200	0.8	1.5	7.3
Sep 88	< 200	0.5	0.7	6.6

Soil water data from the lysimeter soils are presented below for loam and peat.

Date of sample	Concentration in soil water, mBq l^{-1}, loam				
	^{137}Cs	^{239}Pu	^{241}Am	^{90}Sr	pH
Nov 87	525	10	7	24691	8.0
Dec 87	800	14	4	37676	7.6
Jan 88	388	14	4	19096	7.6
Feb 88	289	12	4	11866	7.7
Mar 88	1388	28	7	37392	7.5
Apr 88	1170	14	6	37550	8.2
Aug 88	2155	15	4		7.3

Date of sample	Concentration of soil water, mBq l^{-1}, peat				
	^{137}Cs	^{239}Pu	^{241}Am	^{90}Sr	pH
Nov 87		514	130	5777	6.9
Dec 87	395	307	148	21193	6.1
Jan 88	295	962	95	12298	5.8
Feb 88		640	564	8267	5.8
Mar 88		1728	722	9538	5.9
Apr 88	352	1539	650	11657	5.7
Aug 88		480	268		5.8

Discussion

(i) The concentration ratios determined here for peas are approximately an order of magnitude smaller than those given in the IUR data bank for peas grown under standard conditions. No comparable data are available for carrots.

The most marked effect of the glyphosate treatment was to increase ^{241}Am uptake to peas and carrots (peel especially) grown in loam soil. It is postulated that the higher pH of this soil (7.4 compared to 5.7, 5.9 and 6.1 in peat, clay and sand respectively) resulted in the formation of a stable glyphosate-Am complex which was more available for plant uptake than Am alone. The reduced effect of glyphosate on Am uptake from loam soil to carrot flesh is most likely to be related to the half-life of glyphosate in the soil itself (2 months). For peas, the period from sowing to harvest is 2 months whereas for carrots it is 5 months. Therefore the effect of glyphosate on Am uptake would be reduced during the period of maximum carrot growth (3-5 months).

(ii) Activity concentrations in soil solution from the reclaimed land soil are very low. ^{239}Pu and ^{241}Am activity concentrations in the associated soil (0-50 mm) are 44 and 72 Bq kg^{-1} DM respectively, giving distribution coefficients (K_ds) of 10^3 - 10^4. These data imply that the radionuclides are strongly sorbed to the soil surface and unavailable for plant uptake. There is generally little variation between years and sampling points. A preliminary comparison of destructive and non destructive methodologies shows activity concentrations to be of similar magnitude in the soil solution. High speed centrifugation is likely to remove more firmly held water than the porous cup method where a vacuum of only 0.6Bar is applied. Activity concentrations obtained by this method might be slightly greater therefore than those determined by the centrifuge method. A further comparison will be conducted with the soils from the lysimeters.

Soil water derived from the lysimeter loam and peat soils differ markedly in their pH and radionuclide activity concentrations. ^{137}Cs and ^{90}Sr levels are higher in loam solutions, whilst ^{239}Pu and ^{241}Am are greater in the peat. This has obvious implications for radionuclide uptake to the crops growing in these lysimeter soils. The radiochemical results from the 1988 barley harvest are awaited with interest.

(i) To complete the investigation into the effect of glyphosate on the uptake of radionuclides to crops. This will involve supplementary laboratory experiments to determine the effect of glyphosate on radionuclide availability in the four soil types used in the experiments.
(ii) To complete the investigation of the time dependent transfer of ^{239}Pu, ^{241}Am, ^{90}Sr and ^{137}Cs into crops. The 1989 cabbage crop will be harvested in April and a carrot crop sown and harvested in December.
(iii) To continue work on radionuclides in soil solution, both under lysimeter conditions and in the field. It is hoped from these data to be better able to predict radionuclide availability and uptake to crops.

(Forthcoming)

'The effects of the herbicide glyphosate on the uptake of radionuclides by crops' IUR Workshop, Grimselpass, Switzerland. 24-26 May 1989.

'The applicability of soil solution data in reducing the variability in soil to plant transfer factors' CEC Passariano, Udine, Italy. September 1989.

Title of the project no.: 2

The speciation of radionuclides in plants and foodstuffs and the influence of this on their gastrointestinal uptake

Head(s) of project: Dr D S Popplevell

Scientific staff: Dr R A Bulman, Mr G J Ham, Dr J D Harrison, Dr G P L Naylor, Miss L S Shelton, Mr G Szabo

I. Objectives of the project:

The objectives of the project are to investigate the chemical forms of the radionuclide contaminants of foodstuffs, and the bearing that these have on their gastrointestinal uptake.

II. Objectives for the reporting period:

The objectives for the period were to:-

(i) continue investigations into the chemical forms of radionuclides in foodstuffs and biological fluids;

(ii) continue with the volunteer experiments to measure the absorption of Np and Cm administered as the citrate complexes;

(iii) conduct animal experiments to study the gastrointestinal absorption of radionuclides;

(iv) develop analytical biochemical techniques to characterize both high and low molecular weight binding species for actinides.

III. Progress achieved:

(i) Isotachophoresis (ITP), described in section (iv) has been used to assess the complexing potential of unknown anions present in foodstuffs for species such as Pu(IV). In particular ITP has been used to monitor the nature of the complexing agents which arise on simulated digestion of potatoes, a foodstuff which has been used in investigations of the nature of the gut uptake of plutonium. At least two species of anions, as yet uncharacterized, have been detected by ITP and from their migration characteristics they should be considered as potential complexing agents for Pu (IV). Confirmation of this potential has been obtained by simple gel filtration studies which have demonstrated the formation of low molecular weight complexes with Pu(IV). From ITP investigations of the action of phytase on phytic acid, which has earlier been tentatively advanced as the binding agent which mediates the uptake of Pu(IV) across the gut wall, it would appear that these two unknown anions are not degradation products of phytic acid.

From an examination of solutions of phytic acid by ITP it has been possible to detect a species of anion which must be considered a potential complexing agent for Pu(IV). In view of this observation caution must be exercised when investigating the fractional absorption of Pu(IV) phytate if the phytic acid is a commercially available form, as it cannot be excluded that Pu(IV) is complexed by the trace contaminant. The identity of this trace contaminant is not yet established.

Confirmation of the nature of the speciation of radionuclides is being sought by high performance liquid chromatography, HPLC. However, the analytical techniques still require further refinement.

(ii) The determination of gastrointestinal absorption by means of an intravenous injection experiment as well as an ingestion experiment relies upon the assumption that the actinides entering the circulation from the gastrointestinal tract distribute in the same way as actinides injected intravenously in soluble forms. The first stage of the experiment measured the proportion of intravenously injected actinide that was excreted in the urine over a given period. The following ingestion stage of the experiment used this proportion to extrapolate from the urinary excretion data to give the systemic burden and thence the gut transfer factor.

Neptunium-239 was prepared from neutron irradiated uranyl nitrate solution. Three to four days after irradiation, carrier-free ^{239}Np was separated from the uranium and fission products. Aliquots of the ^{239}Np, evaporated to dryness on steel discs, were counted daily in a gas-flow proportional beta counter. The purity of the ^{239}Np was monitored over about a week, 3 half-lives, and the stock solution was transposed to a form suitable for intravenous injection or ingestion. Pyrogen-free citrate solution isotonic in saline at pH 6.5 - 7.0 was prepared by a medically approved method and calibrated by beta counting. During the course of the experiments, the sources from the original ^{239}Np stock solution were counted for up to 8-10 half-lives to provide evidence, albeit retrospectively, of the purity of the isotope used in each experiment. No evidence was found of any impurity in any of the ^{239}Np batches. The mean half-life of the 6 batches of ^{239}Np was 2.35 $\pm$ 0.01 d, the quoted uncertainty being 2 standard deviations.

Curium-242 was obtained from Harwell Laboratory. The purity of the preparation was checked by alpha and gamma-ray spectrometry. At the time of preparation the material contained approximately 0.00068% of ^{241}Am by alpha activity, or 0.65% by mass. The injection solution was prepared as described for the neptunium solution and was calibrated by alpha counting.

The ^{239}Np and ^{242}Cm were administered at midday, intravenously by a physician, or, in the ingestion experiments, self-administered part-way through the midday meal. Total collections of urine were made for about 10 days. Each sample was analysed for neptunium and curium content.

Three values of the fractional gastrointestinal uptake for curium of 1 to 2 x 10^{-4} have been obtained, and two values of about 2 x 10^{-4} for neptunium. Urinary excretion of ^{239}Np and ^{242}Cm and whole-body retention of ^{239}Np after intravenous injection were very similar for the first two volunteers. In each case, cumulative urinary excretion over about one week accounted for about 40% of injected ^{239}Np and about 10% of ^{242}Cm. For ^{239}Np, comparison with whole-body retention showed that faecal excretion was low. Results for the third volunteer show lower levels of excretion of both ^{239}Np and ^{241}Cm after intravenous injection; about one-half and three-quarters, respectively, of corresponding values for the first two volunteers. The ICRP dosimetric model assumes that for both elements 10% is rapidly excreted. A weighted least squares curve fitting technique was used on the urinary excretion data from the neptunium injection experiments. The data could be fitted to a double exponential curve. The parameters of the second exponential term had large uncertainties and it was possible that there was a third much longer-term component not properly seen in the time scale of the experiment.

With one of the volunteers the neptunium excretion data were incomplete, leading to an estimate of about 10^{-3} for the uptake factor. This value is believed to be erroneous; the ingestion experiment will be repeated with this particular volunteer.

(iii) Experiments are in progress to determine the relationship between increased absorption of physiologically important elements in newborn and young animals and the uptake of related elements. A comparison of the age-dependent absorption of ^{59}Fe and ^{57}Co in rats and guinea pigs will be completed during the next period.

Based on comparisons of whole-body measurements at 14 days after either oral administration or intraperitoneal injection, the absorption of both iron and cobalt in one day-old rats was shown to be virtually complete. By the end of the suckling period at 20 days, absorption had fallen to about 0.7 for iron and about 0.8 for cobalt. Shortly after weaning at 30 days of age, absorption had decreased to about 0.25 for iron and about 0.35 for cobalt. Adult values were about 0.1 and 0.25, respectively. The retention of iron reaching the circulation was much greater than for cobalt, 85 - 95% after 14 days compared with 5 - 10%. Results for guinea pigs are incomplete but indicate a decrease in absorption from about 0.4 in one day-old animals to less than 0.05 in adults. Measurements of iron status in both species are planned.

The retention of radionuclides in the intestinal wall of newborn animals is being studied in rats and guinea pigs. Previous studies have shown high levels of retention of plutonium in the small intestine of newborn rats but much lower levels of retention in newborn guinea pigs. Recent experiments suggest that this is not related to the early consumption of solid food by newborn guinea pigs. Low retention was observed after administration of plutonium to animals on the first day post-partum. Retention in the small intestine accounted for about 2% of the ingested activity at 5 days after administration and 0.5% at 10 days. Autoradiographs showed that retained activity was largely confined to the upper central regions of the villi under the epithelial layer. Concentration in cells suggests uptake by phagocytes. No concentration of activity was observed in lymphoid tissue.

(iv) The nature of the speciation of radionuclides is being investigated by using the modern physico-chemical procedures, high performance liquid chromatography (HPLC) and isotachophoresis (ITP).

The versatility of ITP as an analytical aid lies in its potential to detect complexed cations of a particular species of polyvalent cation which might differ only in the number of complexing anions bound by the cation. At NRPB, ITP is being used to study the chemical forms of the cations of the transuranic elements, or elements, such as niobium, whose chemistry is relatively poorly understood in the presence of biological complexing agents such as citrate. The isotachophoretic method being used at NRPB still requires further refinement but so far it has been used in a preparative manner to show the existence of only one form of Pu(IV) citrate and the nature of Nb(V) when complexed by citrate.

As the procedures used to extract humic substances, now recognized as binding agents of Pu(IV) in soils, disrupt the nature of metal-organic complexes, alternative procedures have been evaluated for the extraction of metal-organic complexes from soil. By trimethylsilylation of soils it has been possible to solubilize the metal-organic complexes present in soil. By using non-aqueous size exclusion chromatography it has been shown that organic complexes of several metallic elements, including plutonium, can be isolated from soils.

As the solubility characteristics of humic substances impose constraints upon investigations of their interactions with Pu(IV) and Am(III), a silica gel upon which humic and fulvic acids are chemically bound has been synthesized to facilitate investigations not only of the uptake of these radiocations but also the desorption. The conclusions which can be drawn at the moment from these studies are : (i) humic acid binds the radiocations avidly; (ii) in ground waters containing dissolved humic and fulvic acids, Pu(IV) and Am(III) will be bound preferentially by humic acids; (iii) shifts in the equilibria of the radiocations from the organic macromolecules to low molecular weight complexing agents are not determined exclusively by the stability of the complex formed between the low molecular weight complex and the radiocation.

IV. Objectives for the next reporting period:

(i) characterise the complexing agents produced by the simulated digestion of potatoes;
(ii) continue with experiments on the gastrointestinal absorption in humans;
(iii) prepare Pu(IV) and Am(III) complexes of the complexing agents and determine their gastrointestinal absorption, in animals; determine gastrointestinal uptake of radionuclides, particularly thorium and polonium, in animals;
(iv) determine the extent to which Pu(IV) and Am(III) bound to humic and fulvic acids become available for uptake into plants. Use these data to model the uptake of these radiocations from soils into plants.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Gastrointestinal absorption of neptunium and curium in volunteers. J D Harrison, D S Popplewell, G Etherington and G J Ham. Radiological Protection Bulletin 93, 11-14 (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-B-040-B

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Rue Charles Lemaire 1
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Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 014/31.18.01

Title of the research contract:

Behaviour of radionuclides in terrestrial and freshwater environments.

List of projects:

1. Technetium behaviour and toxicity in mammals.
2. Comparative study of the radioecology of the continental water of the Meuse and Rhône basins.
3. Dynamic environmental cycling of HTO/HT/OBT. Experimental studies and modelling.

Title of the project no.: 1

Technetium behaviour and toxicity in mammals

Head(s) of project:

J. Vankerkom

Scientific staff:

C. Vandecasteele, M. Van Hees, M. Lambiet-Collier, J. Maisin, R. Kirchmann (advisor), G. Gerber (advisor)

I. Objectives of the project:

The project aims to define the uptake and metabolism of technetium in mammals under different conditions of exposure and to determine the chemical and radiological effects of technetium in developing and adult organisms in order to assess the risk of technetium uptake by man.

II. Objectives for the reporting period:

The objectives for this period were to study the toxic effects and the possible cancerogenicity of chronic oral administration of technetium in rats, to investigate the transfer to the foetus or to the new born lamb of technetium administered to the ewe during pregnancy and lactation and finally to complete the analysis of the data regarding the long-term retention in rat.

III. Progress achieved:

An experiment started in 1987, aiming to study the retention of technetium in rat after 5 weeks of chronic administration of contaminated food, has been completed this year. The last groups of rats (four replications in each group) were sacrificed, providing data on the long-term retention (up to 140 d) and the technetium still present in the animal organs has been measured by liquid scintillation counting. These last points allow a more accurate estimation of the excretion parameters by non-linear least square methods, considering a two compartments model. The parameters estimated for the different organs considered are given in the following table.

ORGAN	FIRST COMPARTMENT		SECOND COMPARTMENT	
	CAPACITY (Bq/MBq/g food)	HALF-TIME (d)	CAPACITY Bq/MBq/g food)	HALF-TIME (d)
Thyroid	4045.4 ± 295.3	0.74 ± 0.18	1249.4 ± 196.2	42 ± 15
Heart	6.8 ± 0.3	0.50 ± 0.11	1.1 ± 0.1	58 ± 21
Spleen	9.2 ± 0.7	0.51 ± 0.21	3.0 ± 0.4	59 ± 20
Kidneys	141.5 ± 1.6	1.03 ± 0.02	24.2 ± 1.4	30 ± 2
Liver	39.5 ± 0.2	0.82 ± 0.01	2.7 ± 0.1	25 ± 2
Pancreas	8.1 ± 0.2	0.51 ± 0.05	0.8 ± 0.1	60 ± 19
Lungs	15.1 ± 0.3	0.62 ± 0.05	1.0 ± 0.2	56 ± 24
Brain	1.3 ± 0.1	0.24 ± 1.28	0.3 ± 0.1	92 ± 58
Muscles	not available		0.6 ± 0.0	52 ± 6
Bones	10.3 ± 0.6	1.48 ± 0.26	2.9 ± 0.4	78 ± 28
Fat	4.9 ± 0.1	0.67 ± 0.03	1.2 ± 0.1	122 ± 46
Salivary gl.	10.5 ± 0.1	0.47 ± 0.08	1.2 ± 0.2	45 ± 16

Depending on the organ or tissue considered, the half-times estimated for the first compartment range from 6 hours (in brain) to 36 hours (in bones) ; the half-times calculated for the second one range from 25 d (in liver) to 122 d (in fat). In kidneys and liver, a third compartment of intermediate half-time (respectively 3.6 and 2.5 d) could be estimated.

The study on the long-term toxicity and possible cancerogenicity of chronic oral feeding with Technetium contaminated food has been initiated in May 1988. Females Wistar rats from the R/Cnb inbred strain were mated. Twenty-eight positive females were divided into two groups (treated and control). The rats from the treated group was fed contaminated food (10 µg Tc-99/g food). At weaning 56 young rats (28 males + 28 females) from each group were selected and the young rats from contaminated mothers were kept on the same contaminated food while the others received the same food without Tc. Moreover all young rats are given a goitrogen (propylthiouracyl 0.04%) in the drinking water until the age of 6 months. At the age of 8 months one third of the animals in each group will be slaughtered for microscopic examination of their thyroids. The remaining animals will maintained in the same conditions and will be sacrificed 4 months later unless the results of

the observations on the thyroids from the first groups slaughtered require a modification of the experimental protocol.

A preliminary experiment on the transfer of technetium in ewe and its progeny was performed. The behaviour of technetium (Tc-95m) was compared to the behaviour of three other radionuclides of importance under accidental conditions : cerium (Ce-141), ruthenium (Ru-103) and silver (Ag-110m). These four radionuclides were administered to two ewes at the end of gestation (three days before lambing), to the first one by injection into the rumen and to the other one intraperitoneally ; the same radionuclides were injected into the rumen of two other ewes six days after delivery. The activities injected were of 27 MBq for Ce-141, 26 MBq for Ag-110m, 25 MBq for Ru-103 and 14 MBq for Tc-95m. One of the ewes injected into the rumen after delivery was placed on a metabolic cage to allow collection of urine. The excretion of technetium by urine appears to be very rapid (maximum concentration in the sample from the day of dosing) compared to that of other radionuclides (maximum concentration in the sample from the second day). The available data allow to estimate the parameters of a first compartment characterised by a half-time comparable for all nuclides (about 0.7 d) and reveal the existence of a second compartment of which the parameters could not be determined. Blood samples were taken from the first day after dosing up to 32 days later. The activities measured in blood samples collected the first day after dosing show a comparable transfer from the GIT for Tc, Ru and Ag (in the range of 0.01%/l of the administered dose) ; the transfer of Ce is about two orders of magnitude lower. The elimination of Tc from blood is relatively rapid, as for Ce, (half-life of the first compartment identified $\simeq$ 1 d) compared to the elimination of Ru and Ag (half-life of the first compartment $\simeq$ 5-10 d).
Most contaminated organs from ewes are thyroid, kidneys and liver in the case of technetium ; liver in the case of silver and cerium ; kidneys, liver and genital organs in the case of ruthenium. In lambs from all treatments higher technetium concentration are found in thyroid ; silver is mostly accumulated in liver and to a lower extent in brain ; cerium is found in bones and in liver and ruthenium in liver and kidneys and to a lower extent in pancreas.

IV. Objectives for the next reporting period:

Follow up of the experiment on the toxic effect and possible cancerogenicity of chronic oral administration of technetium-99 in rats. Study of the transfer in utero and via lactation in monogastric (rats and minipigs) and polygastric (sheep) mammals.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Laboratoire de Physiologie Végétale, Univ. Catholique de Louvain, LLN.
Laboratorium voor Colloïdale Scheikunde, Katholieke Univ. Leuven, Leuven.
Laboratoire de Biochimie, Univ. de Nantes, Nantes (France)

VI. Publications:

Vandecasteele C.M., F. Capot, J.-P. Dehut, J.-M. Mousny and C. Myttenaere (1987)
"Technetium fate in irrigated rice field", Proc. Seminar on the "Cycling of long-lived radionuclides in the biosphere : observations and models", Madrid (E), September 15-19, 1986, CIEMAT, Vol 2 : 207-225.

Dehut J.-P., C. Van Hove, C.M. Vandecasteele and C. Myttenaere (1987)
"Technetium cycle in the environment : redistribution of bioincorporated Tc in Azolla sp. within the different compartment of a terrestrial ecosystem", Proc. Seminar on the "Cycling of long-lived radionuclides in the biosphere : observations and models", Madrid (E), September 25-19, 1986, CIEMAT, Vol 2 : 227-238.

Title of the project no.: 2

Comparative study of the radioecology of the continental water of the Meuse and Rhone basins

Head(s) of project:

C. Vandecasteele

Scientific staff:

P. Govaerts, E. Fagniart, E. Bonnijns, J.C. Micha, J.P. Descy, A.Gillet, R. Kirchmann, J. Lambinon, M. Meurice-Bourdon, C. Myttenaere, L. Sombré, J. Smitz, E. Everbecq, J. Remacle, G. Beuken, H. Declercq-Versele, J.L. Havaux

I. Objectives of the project:

This project is part of a coordinated research programme involving the CEN/SCK (Mol-Belgium) associated to several Belgian laboratories and the CEN/CEA (Cadarache-France). The general objective of this programme is to gain a better understanding of the behaviour of radionuclides released into freshwater ecosystems and to gather a more accurate knowledge on their transfer to man through the food chains. The ultimate objective is to build a general transfer model in freshwater systems based on field measurements and observations, completed by laboratory experiments at various levels. Two waterway systems are investigated : the Meuse basin (Belgian contribution) and the Rhone basin (French contribution) ; the data and models obtained for both rivers will be compared.

II. Objectives for the reporting period:

- Acquisition of site specific data regarding the contamination levels in fish.
- Completion of the studies on Cs-134 as CsCl uptake by diatoms and starting of the transfer experiments with diatoms and real effluents from a PWR. Preliminary studies on the role of bacteria on the cycle of the radionuclides in fresh water ecosystems.
- Improvement of the experimental model of a river section and starting of the experimental work on the transfer between liquid and solid phases. Study on the transfer fish (prey) - carnivorous fish.
- Modelling of the experimental results and development of the general radioecological model.

III. Progress achieved:

1. Field studies :

Two sampling campaigns have been organised in 1988 (the first one during the spring months and the second one in October). Fishes (roach and perch) were collected at three stations on the Meuse river : Waulsort (up-stream from Namur), Ampsin-Neuville (down-stream from the Tihange nuclear plant) and Lanaye (down-stream from Liège). For comparison, samples from the same fish species were taken from a fish-breeding pond in the region of Chimay and samples of roach and pike-perch were caught in the old channel from Charleroi to Brussels. All the fish samples were prepared and sent to the IHE for gamma spectrometry counting.

2. Laboratory studies :

The studies of the accumulation of Cs-134 (as CsCl) by stationary cultures of Cyclotella meneghiniana, a diatom representative of the Meuse phytoplancton have been completed. The results confirm that the uptake of this radioelement is very rapid and that a plateau is reached after a few hours. The transfer factors calculated, based on the initial radioactivity level of the culture medium, range from 1000 to 10,000.

Experiments designed to check the bioavailability of the chemical forms of radiocobalt and radiocaesium in the effluents of the three units of the Tihange nuclear plants for this diatom has been initiated using both the synthetic medium and a medium made of Meuse water enriched with phosphates, nitrates and silicates. These experiments are run to allow the comparison of the bioavailability of these radionuclides from the effluents and that of chloride forms.

The study on the transfer of radioactivity between water, bacteria and sediment has been started in 1988 in order to understand and quantify the role of the bacterial flora in the fixation and recycling of the radioelements in the water column and at the sediment level. Experimental methods and techniques were tested and preliminary experiments were carried out with stable cobalt ($CoCl_2$). Mixed population of bacteria isolated from the Meuse river were first grown in Meuse water enriched with cobalt and starch and peptone as a carbon substrate ; they were then centrifuged and poured in dialysis bags (in presence or absence of sediments) in raw Meuse water under continuous agitation. First results have shown that 60% of cobalt initially accumulated was released by the bacteria within three days regardless of the presence or absence of sediment.

The construction of the experimental model of a river section has been terminated. It consists of a main sub-unit representing the river section (1.6m x 0.5m x 0.5m) filled with Meuse water and a 6 cm sediment layer (30 l) taken from the Meuse river. It is fed with water from a tank with a V-shaped outfall (estimation of the flow rate) and flows into a collecting reservoir. The water is circulated by a pump (maximal flow rate of 10 l/s). The flow in the river section is made laminar by forcing the water through two honeycombs placed at the extremities of the simulated river section. The water (600 l) contained in the whole system has been contaminated with 6 MBq of Cs-137 as CsCl. Water samples and sediment cores were taken regularly for radioactivity measurements. Moreover, water samples were passed through 0.5 µm filters to separate suspended matters. It was observed that the contamination level in the filtrated water decreased progressively and reached an equilibrium value corresponding to 23% of the initial radioactivity after 5 weeks.

At this time 75% of the radiocaesium is accumulated in the bottom sediment and 2 to 3% are associated with the suspended matters (2.77 g/ml). The estimation of the distribution coefficients gives a Kd value of 400 l/kg for the bulk of the bottom sediments while the value for the suspended matters is about 100 times higher. These results are comparable to the observations carried under natural conditions and allow to consider our experimental model as a satisfying representation of a real river ecosystem.
The studies on the transfer from a fish (prey) to a carnivorous fish have been postponed to 1989.

3. Modelling :
The modelisation of the radionuclides transfer through the food chains has been carried on using experimental results on the transfers between water, algae and mussels, obtained in the framework of this contract. First results show that the classical approach using transfer factors has limited possibilities for predictions compared to the use of deterministic models.
The development of the general model of the radionuclides behaviour in a river ecosystems has been carried on. The efforts in this field were devoted to the simulation of highly non-stationary releases. These simulations allow to check the ability of the model for predictions under accidental conditions. The model that has been developed is able to simulate the dynamic of an accidental contamination of an aquatic system and to emphasise the storage and recycling ability of certain compartments (namely the sediment compartment).
The introduction of contamination data for fish in the data bank was continued.

IV Objectives for the next reporting period

The studies on the transfer to diatoms of Cs and Co from effluents from a PWR will be carried on. Transfer experiments will be realised on mixed algal populations isolated at different seasons from the Meuse phytoplancton. Studies on the role of bacteria in the cycle of radionuclides in a river system will be continued. Studies on the transfer of radiocaesium from a fish (prey) to a carnivorous fish will be undertaken.
The modelling of experimental results will be carried on and the results will be incorporated in the general modelisation of the behaviour of radionuclides in a river system (application to the Meuse basin).

V Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Unité d'Ecologie des Eaux Douces, Facultés Notre Dame de la Paix, Namur; Laboratoire de Physiologie Végétale, Univ. Catholique de Louvain, LLN ; Laboratoire de Radioécologie, Univ. de Liège, Sart-Tilman (Liège) ; Unité de Modélisation Mathématique des Eaux Intérieures, Univ. de Liège, Sart-Tilman (Liège) ; Institut d'Hygiène et d'Epidémiologie, Bruxelles ; The Centrale Nucléaire de Tihange (Intercom) collaborates actively to this project ; Laboratoire d'Etude de la Pollution des Eaux, CEN-CEA Cadarache (France).

VI. Publications:

CEN/SCK (1989)
"Etude comparée de la radioécologie des eaux continentales des bassins mosan et rhodanien, contribution des laboratoires belges", Rapport Technique d'Avancement 1987, in press.

Gillet A. and Micha J.C. (1987)
"Biologie et radiocontamination de trois espèces animales (Dreissena polymorpha (P.), Rutilus rutilus (L.), et Perca fluviatilis (L.)) représentatives de différents maillons trophiques de l'écosystème Meuse", Ann. Assoc. Belge Radioprotection, 12 (2-3) : 139-156.

Descy J.P.(1987)
"Etudes écologiques de la Meuse en relation avec les rejets des centrales nucleaires", Ann. Assoc. Belge Radioprotection, 12 (2-3) : 127-138.

Sombré L., Carraro S. and C. Myttenaere (1987)
"Transfert du Cs-134 dans une chaîne alimentaire d'eau douce simplifiée : eau - algue verte (Scenedesmus obliquus) - mollusque filtreur (Dreissena polymorpha)", Ann. Assoc. Belge Radioprotection, 12 (2-3) : 205-230.

Sombré L., Carraro S. and C. Myttenaere (1987)
"Contamination d'une algue verte d'eau douce (Scenedesmus obliquus) par des radionucléides typiques des rejets d'une centrale PWR : culture dans un turbidostat", Ann. Assoc. Belge Radioprotection, 12 (2-3) : 157-170.

Smitz J.S., Everbecq E. and Coméliau B. (1987)
"Modélisation du transfert du Cs-137 de l'eau vers le phytoplancton et simulation des expérimentations de laboratoire", Ann. Assoc. Belge Radioprotection, 12 (2-3) : 171-204.

Zeevaert Th, Fieuw G., Kirchmann R., Koch G. and Vandecasteele C.M. (1987)
"Assessment of the dose to man from the sediments of a river receiving radioactive effluents released by a waste treatment facility", Ann. Assoc. Belge Radioprotection, 12 (2-3) : 247-286.

Sombré L., L. Foulquier et C. Myttenaere (1988)
"Simulation de la contamination d'un écosystème dulcicole par du radiocésium rejeté en conditions accidentelles", IVème Symp. Int. de Radioécologie de Cadarache sur l'"Impact des accidents d'origine nucléaire sur l'environnement", Cadarache, 14-18 mars 1988, CEA Cadarache, Tome 1 : C99-C106.

Sombré L. (1988)
"Essai de modélisation du transfert du radiocaesium (Cs-134 et Cs-137) dans une chaîne alimentaire d'eau douce simplifiée : eau-algue verte (Scenedesmus obliquus)-mollusque filtreur (Dreissena polymorpha)-poisson (Barbus barbus)" Thèse de doctorat en Ecologie (Radiohydrobiologie), Faculté des Sciences Saint-Charles, Université de Provence, Aix-Marseille I, 152pp.

Smitz J.S. and E. Everbecq (1988)
"Accidental releases of radionuclides in waterways" CEC Workshop on "Recent advances in reactor accident consequence assessment", Roma, 25-29 January 1988, in press.

Title of the project no.: 3

Dynamic environmental cycling of HTO/HT/OBT.
Experimental studies and modelling.

Head(s) of project:

S. Bonotto (Source term)
E. Fagniart (dynamic models in soil-plant systems)
C. Vandecasteele (Tritium and C-14 transfer in mammals)

Scientific staff:

J. Vankerkom, M. Van Hees, M. Mergeay (advisor), M. Meurice-Bourdon, R. Kirchmann (advisor), G. Gerber (advisor)

I. Objectives of the project:

The general objective of this project is to gain a better understanding of the environmental behaviour of tritium and carbon-14 by an integrated approach involving modellers and experimenters. Three aspects of the environmental cycle of tritium and carbon-14 will be considered :
- the source term : study on the formation and release of OBT by degradation of resins used for water purification in PWR and on the possible contribution of microorganisms to OBT formation,
- The modelling of H-3 and C-14 behaviour in a soil-plant system : laboratory and field experiments are planned to gather information on the physical and biological mechanisms of HT oxidation and incorporation by plants,
- the transfer of C-14 and tritium under various chemical forms to mammals (mono- and polygastric) is studied in order to predict the behaviour of tritium and C-14 labelled organic molecules in man.

II. Objectives for the reporting period:

Determination of the metabolic activity and life span of yeast cells in a simulated resin circuit of a PWR. Study of the microbial population living in the primary loop of a PWR.
Study under controlled conditions of the mechanisms involved in the HT incorporation by plants.
Study on the transfer through the food chain of various forms of OBT ; preparation of a preliminary experiment on rat.

III. Progress achieved:

1. Source term :

The red yeast isolated from a derivation from the primary loop of the BR2 (Mol-Belgium) and used as a biological model in this study on the role of microorganisms in the formation of tritiated molecules in the primary circuit of a PWR has been identified as belonging to the genus Rhodotorula. Rhodotorula present themselves as ovoidal to elongate cells, reproducing by budding. Grown on rich culture media they synthesise red carotenoid pigments. Its optimal growth occurs at 25-30°C, decreasing toward higher values. No development is observed at 42°C. This yeast is UV resistant.

The ability of these organisms to survive in the purification resin circuit of the primary loop of a PWR has been tested experimentally in a laboratory model. Rhodotorula grown on a rich culture medium have been introduced in the experimental model of the resin circuit previously sterilised and filled with a solution of LiCl and boric acid similar to the solution of the primary circuit of a PWR. The system was thermostatised at 40°C (temperature encountered at the resin level in a PWR) and the solution was circulated during one week. Samples of the solution of the primary loop were taken daily for viable cells counting by plate count agar. An important decrease with time (by a factor 100-300) of the yeast population has been observed that could not been explained by a filter effect of the resin bed. In parallel to the decrease of the yeast population, an increase of a bacterial population probably initially introduced with the non sterile resin was noticed. Most of these bacteria were gram negative.

2. Dynamic models in soil-plant systems :

It has been demonstrated that plants were able to oxidise atmospheric HT into HTO and to incorporate tritium as OBT (see progress report 1987). Experiments were designed to confirm these findings and to try to understand the mechanisms involved in this process. Maize plants grown on Hoagland-Arnon nutrient solution were exposed in a glove box during four hours to an atmospheric HT concentration of 120 MBq/l. Care was taken to wash the HT gas from HTO prior to the injection in the system. The concentrations of HT and HTO in the atmosphere of the glove box were continuously monitored during the exposure as well as the CO2 level (CO2 was reinjected, when needed, to the atmosphere in order to compensate its removal by photosynthesis and to maintain photosynthesis rates to a normal level). The rooting system of the plants was isolated from the atmosphere so that only the aerial parts were directly exposed to HT.

After exposure, the atmosphere of the glove box was replaced by uncontaminated air and the system was open. The plants were removed from their nutrient solution, the roots were rinsed with water and the plants were placed back on a fresh nutrient solution. From that time, solutions and plants were sampled at regular intervals from 1 hour up to 21 days after the end of the exposure. HTO and OBT levels were determined in plant parts and HTO in nutrient solutions. HTO levels measured in the nutrient solution on which the plants were maintained during exposure were lower than the HTO specific activities in the roots water suggesting some excretion of HTO from the roots into the nutrient solution. After replacement of the nutrient solution some HTO was still excreted by the root system.

One hour after exposure, the HTO levels were higher in roots and stems than in leaves. The ratio between OBT (in Bq/g O.M.) and HTO (in Bq/ml) was 0.03 in leaves and stems and twice lower in roots. It may be concluded that HT oxidation happens preferentially in roots and stems while the incorporation of tritium in organic molecules is due to photosynthesis in chlorophyllian tissues. The HTO concentration in the various part of the plants decreased with time as the water was transpirated by the plants and an equilibrium was reached after one week when the HTO concentration in the plant organs was equal to the concentration in the nutrient solution. Two compartments were identified in roots and stems ; the half-time of the first compartment is shorter in roots than in stems and seems to correspond to the water contained in easily displaceable water fractions (i.e. plant vessels) while the half-time of the second compartment is similar in these to parts (about 45 d) and may correspond to less easily exchangeable water (probably associated with plant tissues). Only one compartment (half-time of 23 d) could be identified in the leaves, probably due to the lack of observations during the early period after exposure. The decrease in OBT levels in the different plant parts can be attributed to dilution by growth.

Potatoes (peeled and whole potatoes + parings) were also exposed under the same conditions in order to explained the results obtained after HT exposure under natural conditions (see progress report 1986). After exposure, whole potatoes were peeled. A tissue layer of a thickness corresponding the parings was still removed from both type of potatoes (peeled or not before exposure), then successive rings of 1 cm thickness were sampled. The results are presented in the following table.

TISSUE	POTATOES PEELED BEFORE EXPOSURE		POTATOES PEELED AFTER EXPOSURE	
	HTO (Bq/ml)	OBT (Bq/g O.M.)	HTO (Bq/ml)	OBT (Bq/g O.M.)
1st paring	24616 ± 304	2046 ± 45	1747 ± 30	100 ± 3
2nd "paring"	6888 ± 74	537 ± 15	426 ± 3	48 ± 3
outer ring	4662 ± 67	410 ± 9	256 ± 3	23 ± 4
intermediate ring	1638 ± 20	203 ± 21	83 ± 1	ND
inner ring	865 ± 7	85 ± 7	31 ± 1	ND

ND = below detection limit

The ratio between OBT (in Bq/g O.M.) and HTO (in Bq/ml) was of about 0.1 in each samples. A gradient is observed for both HTO and OBT levels from the outer parts to the inner parts of the potatoes from each treatments. It is noticeable that the potato epidermis constitutes an important barrier to the diffusion of HT to the inner layers, but it is also obvious when comparing the potatoes parings exposed as so to the second parings of peeled potatoes and to the two successive parings of whole potatoes that oxidation of HT arises with a higher rate in the epidermal zone.

3. Tritium and C-14 transfer in mammals :

Tritiated milk was available from a previous experiment of chronic oral HTO administration to a lactating cow (see progress report 1986). This contaminated milk has been skimmed and the skimmed milk was powdered. Various techniques were assayed for the separation of two main constituents of skimmed milk (casein and lactose), in order to define a extraction protocol easy to apply and providing good yields. 15 kg of uncontaminated skimmed milk powder and 5 kg of tritiated milk powder were treated with this technique to produce blanco and tritiated casein and lactose. Tritiated fat has been separated from tritiated milk cream. Commercial butter will be used as blanco fat. Each of these constituents will be administered separately to rats in order to study the uptake, distribution and retention of tritium in proteins, sugars and fat.

IV. Objectives for the next reporting period:

- Study of the fate of Rhodotorula sp. and of its organic constituents after lysis, using yeast cultures labelled with tritium and C-14; determination of the factors responsible for the lethality of red yeasts in the experimental model of the resin circuit.
- Carrying on of the studies under controlled conditions of the processes involved in HT oxidation and incorporation by various plant species.
- Study of the uptake, transfer to the progeny during foetal life and via lactation, distribution and retention of tritium orally administrated as tritiated proteins, lipids and glucides to pregnant and non-pregnant rats.

Production of tritiated potatoes for tritiated starch production.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Laboratorium voor Dieren Fysiologie, Landbouwhogeschool, Wageningen.
SERE-IPSN-DPS, CEA Fontenay-aux-Roses
Niedersächsisches Institut für Radioökologie, Hannover
Institut für Radioagronomie, KFA Jülich
Zentralabt. Sicherheit, Radioökologie, KFK Karslruhe
Laboratoire de radioécologie, ULg Liège.

VI. Publications:

Bonotto S., E. Fagniart, M. Mergeay, R. Kirchmann and M. Meurice-Bourdon "Investigation on the origin of organic tritium present in the effluents from nuclear power plants", CEC Tritium Coordination Meeting, Hannover 13-14 December, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-B-051-NL

Landbouwhogeschool
Agricultural University
Salverdaplein 10
NL- 6709 PJ Wageningen

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J. van den Hoek
Dept. Dierfysiologie
Landbouwhogeschool
Haarweg 10
NL- 6709 PJ Wageningen

Telephone number: 83703/83025

Title of the research contract:

Dynamic environmental cycling of HTO/HT/OBT. Experimental studies and modelling. Incorporation and metabolism of OBT, HT and Carbon 14 in mammals.

List of projects:

1. Dynamic environmental cycling of HTO/HT/OBT. Experimental studies and modelling. Incorporation and metabolism of OBT, HT and Carbon-14 in mammals.

Title of the project no.:

Dynamic environmental cycling of HTO/HT/OBT. Experimental studies and modelling. Incorporation and metabolism of OBT, HT and Carbon-14 in mammals.

Head(s) of project:

Dr. J. van den Hoek

Scientific staff:

Dr. J. van den Hoek, Ir. J.T.M. Koumans,
M.H.J. van den Hoek-ten Have, T.C. Viets, D. Vink.

I. Objectives of the project:

Organically bound tritium (OBT) in foodstuffs may be incorporated directly into organic compounds of various organs and tissues of animals and man. The fraction of OBT which is incorporated, the residence time of the tritiated organic compounds and their location are important parameters for the determination of the radiation hazard of environmental tritium to man. It is an important objective of this project to obtain quantitative data for these parameters which are to be used in the model, developed to describe the kinetics of tritium in the human body. The extent of metabolization of tritium gaz (HT) will be investigated by direct introduction of HT into the animals. Also, the long-term behaviour of Carbon-14 will be studied in animals.

II. Objectives for the reporting period:

A potentially important pathway for the intake of tritium by animals and man is the ingestion of food in which tritium occurs organically bound. The transfer of OBT from mother to fetus during pregnancy and to the young animal during lactation was studied in the miniature goat. Administration of OBT was discontinued at weaning and the animals were sacrificed at various intervals afterwards. Tritium levels were determined in over 25 organs and tissues, and half life values were calculated by regression analysis.

A preliminary experiment was carried out in order to study the metabolic fate of tritiated casein introduced into the abomasum of a lactating goat. The results were compared with those obtained after introduction of casein labelled with C-14.

III. Progress achieved:

1. Methodology

Organically bound tritium (OBT) in feed was obtained by spraying young growing grass with THO. After drying in order to evacuate THO completely, the resulting hay was fed to pregnant minigoats equipped with rumen fistules, during the entire pregnancy period of about 150 days and also during the lactation period of about 80 days. The daily dose was approximately 9.5 µCi (351 kBq). The young animals were sacrificed at 0, 7, 21, 48, 92, 115, 155, 205, 240, 276 and 280 days after discontinuation of OBT administration at weaning. OBT levels were determined in over 25 organs and tissues by combustion followed by liquid scintillation counting.

2. Results

The reduction of tritium activity in a given organ is not only the result of metabolic processes but it is brought about also by new growth which dilutes the existing tritiated tissue. It is necessary to correct for this contribution to the overall decrease of tritium levels in order to separate out the reduction of tritium as a result of metabolism. It turned out to be quite difficult to arrive at a good approximation for this correction, mainly because the growth of a particular organ is only rarely proportional to that of the total body. Since the weight of individual organs at very diffent stages of growth was known as well as the weight of the total animal, it became possible to develop a mathematical procedure which yielded growth correction curves for individual organs.
Therefore the results presented in this report, represent the decrease in tritium activity as a result of metabolic processes only.

3. Discussion

The two animals killed at t=0 -that is after a feeding period of OBT to the mother of about 230 days- showed average tritium levels in 32 organs and tissues of 5.05 nCi (187 Bq) and 4.8 nCi (178 Bq) per gram of dry matter respectively with a standard deviation of about 17%. Different fat tissues such as omentum, subcutaneous and kidney fat, had rather high levels reflecting the high tritium content of milk fat during lactation. Generally speaking, a rather homogeneous tritiation of the body had occurred, amounting to about 0.05% of the daily dose per gram of dry tissue.

The decrease of OBT could be described by a one compartment turnover in some tissues, for example in muscular tissue. However, the turnover rate differed markedly according to the type of muscular tissue. Tritium levels in heart and skeletal muscle, and in the involuntary muscle of the urinary bladder decreased with half lives of 80, 128 and 318 days respectively. A one component decrease of tritium activity was found also in the following organs and tissues: subcutaneous and omental fat (45 days), small intestine (62 d), spleen (71 d), adrenal (92 d), uterus (105 d), abomasum (111 d), large intestine (117 d), testicle (132 d), pancreas (208 d), skin (242 d) and tendinous tissue (2712 d). Two different half lives were obtained for: thymus (5 and 57 days), lymph gland (7 and 85 d), liver (13 and 96 d), kidney (14 and 124 d), lung (23 and 224 d), rumen (42 and 161 d), gall bladder (78 and 239 d) and brain (29 and 328 d). Separate analysis of the white and grey matter of the brain showed the turnover of OBT to be much faster in the grey than in the white matter.

The metabolic transfer of tritiated casein, introduced into the abomasum of a minogoat, into newly formed casein and milk fat, appeared to be quite comparable to that of casein labelled with C-14. The amount of tritium and C-14 in newly synthesized milk fat amounted to about 40% of that in newly formed casein. It illustrates the possibility within the animal organism of using amino acids for very diverse purposes.

IV. Objectives for the next reporting period:

The metabolic transfer of tritiated compounds such as tritiated casein, lactose and possibly milk fat after their introduction into the abomasum of the lactating minigoat will be studied in greater detail, particularly with respect to tritium levels in newly synthesized casein, lactose and milk fat.

Determination of the half life values of OBT in organs and tissues of young minigoats will be further refined by the addition of data from animals sacrificed between 1 and 2 years after discontinuation of OBT administration.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. G.B. Gerber, C.E.C., rue de la Loi, Brussels, Belgium

Ir. R. Kirchmann, Drs. C. van de Casteele, E. Fagniart, Department of Radiobiology, S.C.K.-C.E.N., Mol, Belgium.

VI. Publications:

1. Publications in scientific journals

Van den Hoek, J.

European research on the transfer of radionuclides to animals -a historical perspective. Invited paper presented at the Workshop on "The Transfer of Radionuclides to Livestock", 5-8 September 1988, University of Oxford, U.K.

2. Short communications

Van den Hoek, J,

Turnover of Organically Bound Tritium (OBT) in the young goat after administration of OBT to the mother during pregnancy and lactation. Presented at the EULEP Task Group Meeting, 7-8 November 1988, Chilton, U.K. Abstract published in EULEP Newsletter.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-B-049-B

Institut Royal des Sciences
Naturelles de Belgique, IRSNB
Rue Vautier 29
B-1040 Bruxelles

Head(s) of research team(s) [name(s) and address(es)]:

Dr. D. van der Ben
Département Biologie
IRSNB
Rue Vautier 29
B-1040 Bruxelles

Telephone number: 648.04.75 X286

Title of the research contract:

Behaviour of technetium in the marine benthic environment. Experimental studies and modelling.

List of projects:

1. Studies of the technetium behaviour in sediments, sea water and marine organisms and elaboration of a mathematical model allowing to simulate the behaviour of technetium in marine environment of the Belgian coast.

Title of the project no.: 1

Studies of the technetium behaviour in sediments, sea water and marine organisms, and elaboration of a mathematical model allowing to simulate behaviour of technetium in the marine benthic environment of the Belgian coast.

Head(s) of project:

Prof. D. van der Ben, chef du Département de Biologie à l'Institut royal des Sciences naturelles de Belgique, 29, rue Vautier, B-1040 Bruxelles.

Scientific staff:

S. BONOTTO (C.E.N./S.C.K.-Mol), J.-M. BOUQUEGNEAU and C. DOPAGNE (Univ. de Liège), M. COGNEAU (U.C.L.), S. WARTEL (I.R.Sc.N.B.).

I. Objectives of the project:

- Determination of Tc uptake and distribution in coastal marine organisms and food-chains, and in sediments.
- Tc exchange between sea water, marine organisms and sediments.
- Modelling of Tc behaviour in the marine benthic environment.

II. Objectives for the reporting period:

- Effects of light, darkness and low temperature on the incorporation of ^{95m}Tc in Fucus serratus. Uptake and distribution of Tc in normal and in heat inactivated algae.
- Kinetics of ^{99}Tc uptake by mussels.
- Kinetics of Tc transfer between water, sediments, mussels and fish.

III. Progress achieved:

1.Methodology

- Algae: the effects of light, darkness and low temperature (4°C) on incorporation of ^{95m}Tc in Fucus serratus have been investigated. The uptake and distribution of the isotope in normal and in heat inactivated algae (2 min. in sea water at 50°C), were compared. Distribution in whole algae was visualized by autoradiography by means of intensifying screens and Kodak X-ray Ortho G film. The binding of ^{95m}Tc to organic cell compounds was determined by column chromatography on Sephacryl S-200 labelled extracts.

- Mussels (Mytilus edulis): the uptake and elimination of ^{99}Tc were measured, a) when sea water was contaminated, and b) when food was contaminated. On the other hand, mussels were sampled four times a year at four different places along the Belgian coast, in order to determine the Tc and the heavy metal concentrations.

- Food-chain (water-sediment-mollusk-fish): in order to elaborate a hydrodynamic model, a plexiglass apparatus (fig.1) was made. 260 l of sea water at 12°C were stirred by means of two pumps of 1,5 l/sec. each. 25 l of sediment collected off Zeebrugge formed a 5 cm thick layer. Redox potential, water temperature, concentrations of NH_4, NO_2, NO_3 were regularly measured.

2.Results and discussion

- Algae: Darkness caused a strong reduction (about 80%), and heat inactivation an almost complete inhibition of ^{95m}Tc uptake. In algae kept at 4°C, a substantial reduction (about 70%) was observed in short-term experiments. This reduction gradually decreased after several days; it is well known, indeed, that many brown algae grow in relatively cold waters. Autoradiography showed that ^{95m}Tc was heterogeneously distributed along the thalli of normal and heat inactivated algae. The binding of ^{95m}Tc to organic cell compounds was higher when algae were exposed to light than in darkness. In the end, the results suggest that the uptake and metabolism of Tc in Fucus are controlled mainly by physiological processes.

- Mussels: The ^{99}Tc uptake from contaminated food was much less important (8-20%) than the uptake from contaminated sea water (80-92%). The way in which Tc is eliminated by the mussels suggests the existence of two compartments: one (75% of the accumulated Tc) with a very short half-life of elimination (about one hour), and another with a half-life of about ten days. These results also explain the low Tc concentration factors formerly observed in mussels. The energetic metabolism has little influence on contamination kinetics.

- Food-chain: previous experiments have shown that Tc is mostly fixed by reducing sediments or sediments rich in organic matter. Bacteria play an important part in this process.

First of all, contamination kinetics were studied in the apparatus (fig.2). Water and sediments were regularly sampled. By means of the Mitscherlich equation ($TF=M(1-e-E/B)$, the parameters M=81.3 and B=31.6 could be estimated 60 days after contamination. The equilibrium of the system is reached after 99.8 days (3B/0.95). At this stage, the molluscs (Mytilus edulis) and fishes (Solia vulgaris) are introduced into the system. The bioaccumulation kinetics will then be effected.

—▸— Circuit eau de mer

1. Bac expérimental
2. Bac de sécurité
3. Bac de charge thermostatisé
4. Départ circuit eau de mer
5. Pompe
6. Vanne 2 directions (réglage débit)
7. Rotamètre
8. Entrée circuit
9. Tubes en "aid d'abeilles"
10. Niveau eau de mer
11. Sédiment
12. Sortie
13. Niveau inférieur possible
14. Vanne, réglage débit de sortie
15. Stérilisateur UV (15W)
16. Ecumeur
17. Contacteur, contrôle trop-plein
18. Groupe frigorifique
19. Serpentin de refroidissement
20. Liquide de refroidissement
21. Parc à moules, poissons

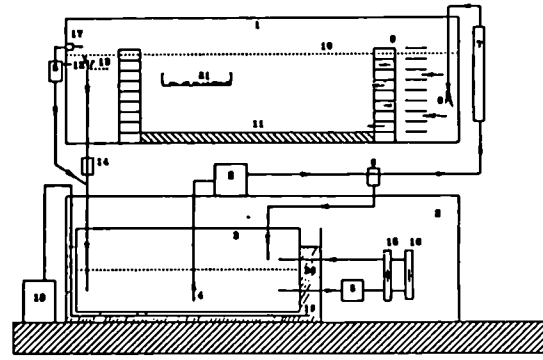
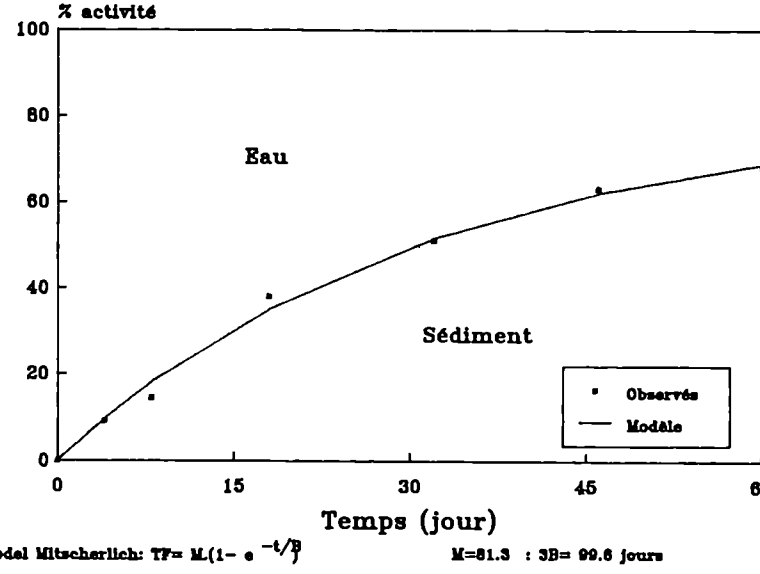


Fig. 1. Schéma expérimental
modèle "Mini-Mer"

Fig. 2 Cinétique de contamination du sédiment



Model Mitscherlich: TF= M.(1- e $^{-t/B}$) M=81.3 : 3B= 99.6 jours

IV. Objectives for the next reporting period:

Modelling of Tc exchange between sea water, sediments and marine organisms.
Writing of the final report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

S. BONOTTO, D. VAN DER BEN, F. CAPOT, J.-M. BOUQUEGNEAU and M. COGNEAU. Technetium in coastal environments: field observations and laboratory experiments. In: U. SEELIGER, L.D. de LACERDA, S.R. PATCHINEELAM (Eds.). Metals in coastal environments of Latin America, pp.222-236. Berlin 1988.

C. HURTGEN, G. KOCH, D. VAN DER BEN and S. BONOTTO. The determination of technetium-99 in the brown marine alga Fucus spiralis collected along the Belgian coast. The Science of the Total Environment, 70, 131-149, 1988.

M. LICOT, J.-M. BOUQUEGNEAU and C. DOPAGNE. Accumulation of technetium by Phaedactylum tricornutum BOHLIN in culture. Océanics, 14 (4), pp.525-531, 1988.

H. FLOROU, M. COGNEAU, Z. MOUREAU, V. ROBBRECHT, D. VAN DER BEN and S. BONOTTO. Incorporation of Tc-95m in the brown Macroalgae Fucus serratus and Fucus spiralis under experimental conditions. Rapp. Comm. int. Mer Médit., 31 (2) 247, 1988.

D. VAN DER BEN, C. VANDENHOUTEN, C. KAREZ, S. PUISEUX-DAO, W. BAEYENS and S. BONOTTO. Uptake, distribution and biological effects of Cadmium in the unicellular marine Alga Acetabularia acetabulum. Rapp. Comm. int. Mer Médit., 31 (2) 183, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Studiecentrum voor
Kernenergie, SCK/CEN
Rue Charles Lemaire, 1
B-1160 Bruxelles

Contract no.: BI6-B-050-B

Head(s) of research team(s) [name(s) and address(es)]:

Prof. O. Vanderborght
Radionuclide Metabolism Section
SCK/CEN
Boeretang 200
B-2400 Mol

Telephone number: 014/31.18.01

Title of the research contract:

Bioavailability of transuranium nuclides in aquatic environments.

List of projects:

1. Biological availability of transuranics in aquatic ecosystems.
2. Speciation of transuranics in aquatic environments.

Title of the project no.:

Biological availability in aquatic ecosystems.

Head(s) of project:

O. Vanderborght

Scientific staff:

J. Vangenechten, S. Van Puymbroeck, O. Vanderborght, S. Vets

I. Objectives of the project:

This project aims to identify the routes and kinetics of uptake of transuranics (americium and curium) in freshwater animals. The uptake and storage of curium and americium in various freshwater animals will be assessed. The role of the hepatopancreas in crustaceans (esp. Astacus leptodactylus) for uptake and storage will be studied, together with the subcellular distribution in target organs. Furthermore, the uptake process of both americium and curium via the gills in freshwater fish (esp. Salmo gairdneri) will be studied. The project further aims to study the mode and kinetics of excretion of incorporated forms through the excretory system of the animals. In this way determination of turn-over rates will become possible.

II. Objectives for the reporting period:

The objective for this reporting period has been:

1. to study the uptake of curium in various freshwater animals as compared with uptake of americium,
2. to identify the role of natural organic materials in the water (humic acids) in curium and americium uptake by freshwater animals,
3. to study the uptake routes and kinetics of curium and americium via the gills of freshwater fish.

III. Progress achieved:

Experiments were performed to determine the bioavailability of curium-244 to the pond snail (Lymnaea stagnalis), freshwater crustaceans (gammarids) and rainbow trout (Salmo gairdneri).

Uptake in snails

Uptake experiments performed in the pond snail showed concentration factors for curium-244 ranging from 200 to 300. Around 84% of the curium content in the snail was measured in the soft tissues. Only 16% of the radionuclide was fixed on the shell. High concentration factors were measured in the hepatopancreas, ranging from 4000 to 7000. These data for snails indicated that curium-244 more than americium-241 became assimilated in soft tissues leading to high concentration factors in the organs. These observations may have considerable radioecological consequences especially because dose assessment models for risk evaluation for curium often extrapolate the data of americium.

Uptake in fish and gammarids

Laboratory experiments on the uptake of curium and americium in rainbow trout showed concentration factors after 7 days of uptake ranging on average from 0.12 to 0.47 for Cm and from 0.06 to 0.42 for Am, according to the water type used. The differences in concentration factor as mentioned, were due to the presence or absence of humic matter in the water leading to a sharp decrease in transuranium uptake when humic acids were present (see also part 2 on the physico-chemistry of curium and americium). Concentration factors after 10 days of uptake in Gammarids averaged 200. Former experiments with Gammarids on americium-241 accumulation revealed concentration factors as high as 700 after 8 days of uptake.

Role of humic acids in transuranium uptake

Natural organic acids, humic acids for example (HA) are known to form com plexes with transuranic elements (see part II of this contract). Complexation of americium or curium to humic acids may lead to different uptake of the transuranics in freshwater organisms. Accumulation of curium-244 in snails was 1.6 times lower in waters with humic acids (10

mg HA/l). The relative distribution of the radionuclide among the snail's tissues did not vary with the water type. Accumulation of americium and curium in freshwater fish over a period of 7 days, yielded concentration factors in whole fish which were respectively 7 to 4 times lower in water with 10 mg HA/l. Concentration factors in tissues were 5 to 30 times lower for animals in water with organic acids. All observations point to the importance of the presence of organic material in water for decreasing the bioavailability of transuranium radionuclides.

Mechanisms of uptake

For modelling purposes it is important to know what part of the transuranic is ultimately taken up by freshwater animals. This is the so-called bioavailable fraction. We investigated one of the possible biological mechanisms by which americium and curium may be taken up in freshwater fish.

These studies aim to identify suitable physico-chemical configurations of the americium and curium species in the water which are likely to be transported in the animals via the studied uptake routes. One of these uptake routes in fish may be the Ca-channel in the epithelium of the gills. In our studies we blocked the Ca-channels in trout gills by addition of lanthanum to the water. We simultaneously studied the accumulation of curium and americium in the liver. The accumulation of americium in the liver after 24 h of exposure in lanthanum rich water was decreased with 67% whereas Ca influx in the blood under these conditions was decreased with 70%. It was concluded from these experiments that americium accumulation in rainbow trout for a large part occurred via the La-sensitive Ca-channels in the gills. These results indicate that americium is principally taken up by the fish in its ionic form. It is further argued that the calcium concentration in the natural environment, which determines the calcium influx kinetics, may significantly determine the accumulation of transuranic element like americium.

IV. Objectives for the next reporting period:

a. subcellular metabolism of curium will be compared with that of the other transuranics.

b. the pathways of entering of freshwater animals will be further experimentally tested.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. K. Simkiss, dept. of zoology, University of Reading, Reading, UK.

VI. Publications:

J. Bierkens, J.H.D. Vangenechten, S. Van Puymbroeck, O.L.J. Vanderborght. 1987. The uptake of 241-Am by the crayfish Astacus leptodactylus following intragastrical and intracardial injection. In: Proc. Seminar on the cycling of long-lived radionuclides in the biosphere: Observations and models. Madrid, Spain, september 1986, ECSC-EEC-EAEC, Brussels-Luxembourg.

J. Bierkens and O.L.J. Vanderborght. Speciation and bioaccumulation of transuranic elements in the freshwater environment. Submitted as a chapter in : "Speciation of actinides in the environment" (Bulman, R.A.,Ed.), CRC Press inc. Boca Raton, USA. In press.

J.H.D. Vangenechten, S. Van Puymbroeck and O.L.J. Vanderborght. Curium-244 uptake in freshwater snails. Proc. IVth International symposium of radioecology of Cadarache on the impact of nuclear origin accidents on the environment. March 1988, Cadarache France, CEC-CEA, In press.

J.H.D. Vangenechten, S. Van Puymbroeck and O.L.J. Vanderborght. Curium-244 and americium-241 uptake in freshwater fish. J. Toxicol. and Environm. Chem. In press.

J.H.D. Vangenechten, S. Van Puymbroeck, S. Vets and O.L.J. Vanderborght. Americium and curium permeation through calcium channels in rainbow trout gills. In preparation.

Title of the project no.:

Speciation of transuranics in aquatic environments.

Head(s) of project:

O. Vanderborght

Scientific staff:

J. Vangenechten S. Van puymbroeck S. Vets O. Vanderborght

I. Objectives of the project:

This project aims to correlate the chemical behaviour of americium-241 and curium-244 in freshwater environments with their bioaccumulation in animals. This relation indeed remains uncertain although bioaccumulation of transuranium elements may differ by one order of magnitude according to the water used. The behaviour of the transuranium nuclides will therefore be studied in particular respect to other dissolved and undissolved substances in the water. Parameters as pH-value, natural organic- transuranic interaction, presence of metals will be examined. These studies will be carried out in direct relation to the experiments and findings of project 1.

II. Objectives for the reporting period:

a. Study of the speciation of curium in freshwater in relation to its bioavailability
b. Study of the role of humic acids for curium-244 bioavailability.

III. Progress achieved:

In former experiments, the relation between the speciation of americium-241 and its biological uptake was studied. Water quality characteristics which determine the americium speciation appeared to be pH, HCO3, CO3, and na tural organic acids (Humic Acids: HA). The speciation behaviour of curium-244 was studied during various accumulation experiments in freshwater environments (see also project nr 1).

The curium-244 particulate formation was studied in synthetically prepared solutions with 23 mg Na/l, 2 mg K/l, 1 mg Mg/l, 61 mg HCO3/l, 38 mg Cl/l and 15 mg SO4/l. The amount of the particulate (>0.45 um) curium fraction ranged from 50% to 80% (average 63%) in these solutions. When natural organic acids were added to this water at a concentration of 4 mg C/l, the particulate curium concentration averaged 17% of the total curium concentration and ranged from 8% to 30%. The presence of dissolved organic matter was clearly seen to inhibit the curium particulate fraction. Similar observations were formerly made for americium-241 in the presence of humic acids. It is argued that curium and americium behave in a similar way by forming complexes with the natural organic acids. Under these conditions, curium accumulation in snails was significantly diminished (see project nr 1).

In another experiment on the accumulation of americium-241 and curium-244 in rainbow trout, the same synthetically prepared solutions were used. Both americium and curium formed filterable particles >0.45 um. The amount of particulate americium and curium averaged 65%+-3 respectively 50%+-2. In water with 4 mg C/l organics, the particulate Am and Cm fraction averaged 28%+-2 respectively 29%+-2 of the total concentration. Both americium and curium thus seem to behave comparably in their particulate formation in the presence as well as in the absence of humic acids. In these experiments, americium and curium accumulation in fish was significantly diminished by the presence of humic acids (project nr 1).

In another experiment, the accumulation of americium in freshwater fish via Ca-channels in the gill-epithelium was studied (see project nr 1) in the presence and absence of lanthanum, a calcium-channel blocking agent. Lanthanum by itself also forms particles (about 45% in the experimental solutions with 1 µmol La/l). Under these conditions, the americium particulate fraction was seen to be enhanced, averaging 71% as compared with 56% in control solutions without lanthanum. This may result from adsorption of Am to existing nuclei of lanthanum. This same process of pseudo-colloid formation of americium has formerly been described to occur also in solutions containing iron. From this experiment with lanthanum, it was concluded that americium is taken up for the largest part in its ionic form.

IV. Objectives for the next reporting period:

Speciation in the animals (gut, hepatopancreas, liver) will be related to the biological half-live of the isotopes.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. M. Hoppenheit, Biologische Anstalt Helgoland, Hamburg (Germany).

VI. Publications:

J. Bierkens and O.L.J. Vanderborght. Speciation and bioaccumulation of transuranic elements in the freshwater environment. Submitted as a chapter in: "Speciation of actinides in the environment" (Bulman, R.A.,Ed.), CRC Press Inc. Boca Raton, USA. In press.

J.H.D. Vangenechten, S. Van Puymbroeck and O.L.J. Vanderborght. Curium-244 uptake in freshwater snails. Proc. IVth International Symposium of Radioecology of Cadarache on the impact of nuclear origin accidents on the environment. March 1988, Cadarache France, CEC-CEA. In press.

J.H.D. Vangenechten, S. Van Puymbroeck and O.L.J. Vanderborght. Curium-244 and americium-241 uptake in freshwater fish. J. Toxicol. and Environm. Chem. In press.

J.H.D. Vangenechten, S. Van Puymbroeck, S. Vets and O.L.J. Vanderborght. Americium and curium permeation through calcium channels in rainbow trout gills. In preparation.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Union Internationale des
Radioécologistes, UIR
Association Internationale
Rue Cardinal Cardijn 5
B-4480 Oupeye

Contract no.: BI6-B-052-B

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Aarkrog
Chairman of IUR
Rue Cardinal Cardijn 5 - Bte 18
B-4480 Oupeye

Telephone number: 041/64.25.64

Title of the research contract:

Promotion of research and exchange of information in radioecology.

List of projects:

1. Intercomparison and harmonization of methodologies, identification of future objectives in radioecology, and training and exchange of scientists.

Title of the project no : 1.Intercomparaison and harmonization of methodologies, identification of future objectives in Radioecology, training and exchange of scientists.

Head(s) of project: Dr.A.AARKROG
Chairman of I.U.R.
rue Cardinal Cardijn,5,Bte 18
B-4480 OUPEYE BELGIUM

Scientific staff:
Working Group Leaders : H.Dahlgaard, L.Foulquier, M.Frissel, G.Linsley, C.Myttenaere, J.Van den Hoek

I.Objectives of the project:

To provide support for exchange of information, standardisation and development of experimental research in Radioecology with a view to protect Man from the harmful effects of radionuclides present in the environment.

To identify the specific needs of developing countries in the field of Radioecology.

To promote the formation of young scientists through participation to scientific meetings and visits to advanced laboratories.

II.Objectives for the reporting period:

a) The aim of the working group "Plant to Animal Transfer" is to establish reliable plant to animal transfer factors for various radionuclides, and to evaluate the importance of different parameters which may influence this transfer.
b) The aim of the working group "Soil-to-Plant Transfer Factors" is to derive reliable transfer factors of radionuclides for nuclear safety assessment studies.
c) The aim of the working group Marine Radioecology (MARECO) is to give scientists engaged in marine radioecology studies an opportunity to meet and exchange information and to co-operate on marine radioecological projects.
d) The WG "Radioecology of Continental Waters" aims to review the studies on the behaviour and transfer of radionuclides, particularly from the Nuclear fuel Cycle, in the freshwater ecosystems.

III. Progress achieved:

a) The C.E.C. organized in close cooperation with the NRPB a Workshop on "The Transfer of Radionuclides to Livestock" from 5 - 8 September 1988 at the University of Oxford. Most members of the W.G. "Plant-Animal" were able to attend this Workshop and presented papers amongst which was an invited paper by J.Van den Hoek.
The Workshop gave ample opportunity for informal contacts between the members present.
Scientists from several countries asked to join the Working Group.
b) The Working Group "Soil-to-Plant Transfer Factors" has a very successful year. The number of transfer factors in the IUR data bank increased to more than 6500 values. The number of data derived from Chernobyl contaminated areas increased to about 200. The latter data are very important because they might show why during the first year after the Chernobyl accident in some areas uptake values were higher than expected.

The data bank contains now more data of radionuclides which are not often reported on, such as I, Po, Pb, Ag, Sb. This has been reached while maintaining the high quality standards, i.e. only data of scientist following the recommendations prepared by the working group are included.

The data of the working group from the core of the soil-to-plant transfer factors of the HANDBOOK OF PARAMETER VALUES FOR THE PREDICTION OF RADIONUCLIDE TRANSFER IN THE TERRESTRIAL AND FRESHWATER ENVIRONMENTS which is prepared by the IAEA in cooperation with the IUR and scheduled to appear at the end of 1989.
c) The Chairman of the "Radioecological Assessment Committee" has participated to the second meeting of the VAMP organized by the IAEA (validation of models for radionuclides transfer in the terrestrial, urban and aquatic environments) 28 - 30 November 1988.
The scope of the meeting was to review and advise and assist on future planning of the VAMP within the framework of the terrestrial environment.
The approach to model validation which was suggested by the members of the terrestrial Group was to review in detail the data associated with several processes simulated in the transfer models in order to test if the related concepts and assumptions are consistent with the data. Several key issues were proposed by the participants to the first meeting (June 88) and then by the members of the consultants meeting.
d) During 1988 the IUR, through the Working Group on "Environmental Assessment Modelling", provided support for the attendance of 3 IUR members at the BIOMOVS Workshop in Tokyo, Japan, 7-13 November 1988.
The BIOMOVS project is now entering its final stages and in the course of the next year final reports on the model validation exercices in most of the scenarios should become available.

Several members of the IUR "Environmental Assessment Modelling" Working Group were present at the first meeting of the IAEA's Coordinated Research Programme on the Validation of Models for the transfer of radionuclides in terrestrial, urban and aquatic environments (2-6 May, 1988, Vienna).
In addition, the IUR itself was formally represented at the meeting by Mr.N.Pattenden (UK).
Subsequently following this meeting four Working Groups were established; these cover the following aspects :
a) Terrestrial
b) Urban
c) Aquatic and
d) Multiple Pathways
Prof.C.Myttenaere will chair the Terrestrial Working Group.
e) A Meeting of the WG "Radioecology of Continental Waters" was held in Cadarache (16 March 1988): 34 Scientists from 14 countries participated to this important Meeting.
A lot of data and analysis of Post-Chernobyl situation have been presented and discussed; it was decided to undertake a Synthesis Report in the framework of this WG activities.

IV. Objectives for the next reporting period:

Collaboration with IAEA
A meeting of experts including IUR members (G.Pröhl,(FRG), Y.Ng,(U.S.A.), M.Frissel (Netherlands) and H.Koehler (IAEA) was organized to continue the work on the IUR/CEC/IAEA project to produce a Handbook of Environmental Transfer Data.
The meeting which took place in Vienna 14-18 November 1988 took account of written comments received on the first draft of the document and of the comments made at the IUR Environmental Assessment Working Group Meeting in Athens in October 1987. A further meeting of the group will take place in 1989.

Plans for 1989
a) Originally, a meeting of the W.G. "Plant-Animal Transfer" was planned for April 1989 in Neuherberg (Germany). However, the exchange of experimental results and ideas during the Workshop at Oxford, has led to a postponement of this planned meeting to 1990. This will give the members an opportunity to continue their research activities which will render the next meeting more fruitful.
A summary of the papers, presented at the meeting at Grange-over-Sands, completed by some new data reported at the Workshop at Oxford, will appear shortly.
b) The next meeting of the Working Group "Soil-to-Plant Transfer Factors" will be May 24-26,1989 in Switzerland. On the agenda are: 1) Presentations of recent research, 2) Statistically derived predictor values, 3) The VIth report of the working group (containing besides the forementioned topics also all data available within the data bank), 4) Making available the data bank by means of diskettes suitable for PC'S.
c) The first working group meeting MARECO is planned to take place in connection with the CEC MARINA seminar in Belgium; preliminary date at present: June 13, 1989.
d) It is expected that IUR members will continue to take part in the three projects BIOMOVS, IAEA Model Validation CRP, and the Handbook of Environmental Transfer Data Project.
A meeting of the Working Group "Environmental Assessment Modelling" may be organized in association with the IUR Annual Meeting (plans for this have yet to be announced).
e) In 1989, the Synthesis Report based on data collected at the Cadarache Meeting will be achieved and reviewed at the next WG "Radioecology of Continental Waters" Meeting (planned in october 1989, in CSSR).
The WG Leader (L.Foulquier) will represent IUR at the Congress of the International Society of Lymnology to be held in August 1989, in München (FRG).

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- KfA (Jülich)FRG
- KfK (Karlsruhe) FRG
- CEA (Cadarache,FAR) France
- CEN/SCK (Mol) Belgium
- Lab.of Animal Physiology (Wageningen) NL
- RIVM (Bilthoven) NL
- Oak Ridge National Lab. (TN) U.S.A.
- NRPB (Chilton) U.K
- AERE ,Harwell (Didcot) U.K.
- Swedish Univ.of Agric.Sc. (Uppsala) Sweden
- RISO National Laboratory (Roskilde) Denmark
- Niedersächs.Inst.für Radioökologie (Hannover) F.R.G.

VI. Publications

- Papers presented by IUR Members at the IVe Symposium International de Radioécologie de Cadarache,France (14 - 18 Mars 1988)
  "Impact des accidents d'origine nucléaire sur l'environnement"
  C.E.A. - avec le concours de l'Union Internationale des Radioécologistes.

  - Greca's survey or radiological data for use in accident consequence assessment.
    U. TVETEN (Norvège)

  - The use of multi-criteria-analysis (MCA) for evaluating feasible countermeasures after an accidental release of radioactivity.
    A. SCHENKER-WICKI, H. GROGAN, F. VAN DORP (Suisse)

  - The availability of deposited 137 Cs to man.
    M.J. FRISSEL, F.C.M. MATTERN, R.M.S. DROST (Pays Bas)

  - Mesures de strontium-90 en Suisse.
    C. FRIEDLI, J.J. GEERING, P. LERCH (Suisse)

  - Transfer to farm animals (ruminants) and their products of Cs-134, Cs-137 and I-131 after the Chernobyl accident.
    J. VANKERKOM, M. VAN HEES, C.M. VANDECASTEELE, J. COLARD, R. KIRCHMANN (Belgique)

- Observations portant sur les retombées consécutives à l'accident de Tchernobyl dans la partie nord de la France.
  C. CAPUT, Y. BELOT, J. GUENOT, D. GAUTHIER (France)

- Studies on the translocation of Cs134 from leaves to fruits of apple trees.
  H. KATANA (Syrie), C. BUNNENBERG, W. KUHN (R.F.A.)

- Transfer of some Chernobyl fallout nuclides in the animal-product food chain.
  J. HANDL, A. PFAU (R.F.A.)

- Soil-to-plant transfer studies of nuptunium, plutonium, americium and curium.
  M. PLIMPL, W. SCHMIDT (R.F.A.)

- Uptake of Cs 137 by fungi in relation to their biology in terrestrial ecosystems.
  R. ROMMELT, L. HIERSCHE, E. WIRTH (R.F.A.)

- Cs and K uptake by plants from 66 soils in Bavaria.
  G. SCHALLER, E. WIRTH (R.F.A.)

- Interpretation of soil-to-plant transfer on the basis of soil solution chemistry.
  J.F. LEMBRECHTS, L.R. VAN LOON, J.H. VAN GINKEL, G.M. DESMET (Pays Bas)

- Transfer of radiocaesium deposited after the Chernobyl accident to agricultural plants.
  C.M. VANDECASTEELE, E. FAGNIART, J. COLARD, R. KIRCHMANN (Belgique)

- Foliar uptake of cesium, iodine and strontium by plants.
  P. KOPP, O. OESTLING, W. BURKART (Suisse)

- La fumure potassique : approche d'une contre-mesure applicable au transfert du 137Cs à partir de sols ferrallitiques.
  R. DUCOUSSO, C. GROUZELLE (France)
  C. COLLE , A. GRAUBY (France)

- Tranfer of cesium to grassland crops in the Chernobyl fallout areas in Sweden in 1986 and 1987.
  A. ERIKSON, H. LONSJO, K. ROSEN (Suède)

- Les dépôts radioactifs consécutifs à l'accident de Tchernobyl dans le bassin du Var.
  H. MAUBERT, S. ROUSSEL, R. LION (France)

- The transfer of cesium 134/137 and cobalt 57/60 from soil into plants after simulation of different types of deposition and affected by various element concentrations and soil tillage investigated in lysimeters.
  W. STEFFENS, W. MITTELSTAEDT, V. TOUSSAINT, M. BILO (R.F.A.)

- Comparison of radionuclide deposition to soil and vegetation.
  P.A. CAWSE (Grande Bretagne)
  C. COLLE (France)

- Cesium-137 in soils and its soil-to-plant tranfer rate.
  C. PAPASTEFANOU, M. MANOLOPOULOU, S. CHARALAMBOUS (Grèce)

- Etude du recyclage du Tc99 incorporé dans la matière végétale forestière.
  S. VAN LEAR, K. FONSNY, C. MYTTENAERE (Belgique)

- Simulation de la contamination d'un écosystème dulcicole par du césium rejeté en conditions accidentelles
  L. SOMBRE, C. MYTTENAERE (Belgique)
  L. FOULQUIER (France)

- Experimental comparative study of accumulation and loss of Sr and Cs radionuclides by bottom sediments of the Black Sea and the lower Dnieper.
  G.G. POLIKARPOV, G.E. LAZORENKO (U.R.S.S.)

- L'impact de l'accident de Tchernobyl - Application fondamentale en hydrosédimentologie.
  P. GUEGUENIAT, R. GANDON, Y. BARON, J.P. AUFFRET, M. WARTELL (France)

- The impact of Chernobyl fallout on Mytilus SP. collected from the French coast
  D. CALMET, S. CHARMASSON, G. GONTIER, M.L. DABURON. (France)

- Cs137 and other radionuclides in the benthic fauna in the Baltic Sea before and after the Chernobyl accident.
  P.O. AGNEDAL (Suède)

- Decrease of 90Sr concentrations in aquatic environment of the lower Dnieper in the Black Sea direction
  G.G. POLIKARPOV, V.I. TIMOSHCHUK, L.G. KULEBAKINA (U.R.S.S.)

- Etude comparée de l'impact radioécologique des installations nucléaires et de l'accident de Tchernobyl sur le fleuve Rhône.
  A. LAMBRECHTS, L. FOULQUIER, M. PALLY (France)

- The experimental study of Cs134 behaviour in freswater sediments.
  M.C. VAZ CARREIRO, M.J. MADRUGA (Portugal)

- Interaction of radionuclides with solid phase in the modelling of the migration of radionuclides in surface waters.
  P. BENES (Tchécoslovaquie)

- Evaluation de l'impact radioécologique de l'accident de Tchernobyl en France sur des écosystèmes aquatiques.
  L. FOULQUIER (France)

- Contamination des écosystèmes forestiers par le radiocésium.
  C. RONNEAU, E. FAGNIART, K. FONSNY, P. ANDRE, C. MYTTENAERE (Belgique)
  H. MAUBERT (France)

- Deposition, resuspension and the long-term variation of airbone radioactivity from Chernobyl.
  J.A. GARLAND, CAMBRAY R.S. (Grande Bretagne)

- Plume dispersion and deposition processes of tracer gas and aerosols in short-distance field experiments.
  M. TASCHNER, C. BUNNENBERG (R.F.A.)

- The dynamic radioecological model ECOSYS. A tool for the management of nuclear accident's consequences.
  G. PROEHL, H. MULLER (R.F.A.)

- Deposition, reemission and subsequent radioecological pathway of elementary tritium after its release in the local ecosystem.
  H. FORSTEL, F. FUHR (R.F.A.)

- Radiological Accident in Goiânia, Brazil
  An overview
  A.H. MENDOCA (Brésil)

- Fallout deposition of actinides at Monaco and Denmark following the Chernobyl accident.
  E. HOLM, S. BALLESTRA, J.J. LOPEZ (AIEA),
  A. AARKROG (Danemark)

- C.E.C. Research on environmental consequences of nuclear accidents. Policy and programmes.
  F. GIRARDI, G. GRAZIANI, F. LUYKX, J. SINNAEVE (C.E.E.)

- Impact des retombées provenant de l'accident de Tchernobyl sur les bioindicateurs végétaux utilisés en routine pour la surveillance radioécologique.
  E. VAN GELDER, J.M. LAMBOTTE, J. LAMBINON, R. KIRCHMANN (Belgique)

- On the transport and trajectories of the Chernobyl debris across Canada and the Artic.
  J.C. ROY, S.R. JOSHI (Canada)

- Données sur l'occupation des sols des 21 sites de centrales nucléaires français dans un rayon de dix kilomètres.
  B.E.G.E.A.T. (France)

- Impact et dynamique de la radioactivité provenant de Tchernobyl dans trois bassins versants.
  J. DELMAS, A. GRAUBY, D. CALMET, C. CAPUT, B. DESCAMPS, P. GUEGUENIAT, H. MAUBERT, L. OTTAVI (France)

- Radionuclides behaviour in the Po basin (North Italy) after the Chernobyl accident.
  A.A. CIGNA, G. QUEIRAZZA, L. GUZZI (Italie)

VARIA

- Booklet :Radioecological Research Centres in the World: updated version of "Present Status and objectives in Radioecology" September 1988.
- Information Bulletin of IUR N°8 (1988)
- Bilan des études sur les transferts en eau douce. L.Foulquier. Journée Scientifique du 2 juin 1987 organisée par la Société Français de Radioprotection du Sud-Est.
- The IUR Project on Soil-to-Plant Transfer Factors of Radionuclides expected values and uncertainties. M.J.Frissel and J.Koster in "Reliability of Radioactive Transfer Models" G.Desmet(ED) Elsevier, London,1988.

- Reports of Missions performed by IUR Representatives
- Visit at Chernobyl, 22 March 1988
- Radioecological contributions to the IRPA 7 Congress in Sydney, 10 - 17 April 1988
- Report of IUR's Delegates visit to China, 26 April - 15 May 1988.

III C

NICHTSTOCHASTISCHE WIRKUNGEN IONISIERENDER STRAHLEN

NON-STOCHASTIC EFFECTS OF IONIZING RADIATION

EFFETS NON-STOCHASTIQUES DES RAYONNEMENTS IONISANTS

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Univ. Catholique Louvain-la-Neuve
Halles Universitaires
Place de l'Université 1
B - 1348 Louvain-la-Neuve

Contract no.: BI6-C-187-B

Head(s) of research team(s) [name(s) and address(es)]:

Prof. H. Bazin
Unité d'Immunologie Expérimentale
Univ. Catholique Louvain-la-Neuve
30 Clos Chapelle aux Champs
B - 1200 Bruxelles

Telephone number: 02-764.34.30

Title of the research contract:

Consequences of irradiation on the immune system. Prevention and treatment of its effect.

List of projects:

1. Role of B lymphocytes in chronic radiation damage.

Title of the project no.:

ROLE OF B LYMPHOCYTES IN CHRONIC RADIATION DAMAGE.

Head(s) of project:

Prof. Hervé BAZIN

Scientific staff:

Dr Patrick MANOUVRIEZ

I. Objectives of the project:

Injuries of the immune system are a well known consequence of exposure to ionizing radiations. As the immune response is the results of a series of events which imply a number of cell populations, it is necessary to identify them and to analyse their respective radiosensitivities. The present program is focused on the humoral responses and especially on the B lymphocytes which can be divided in various subpopulations, depending on their membrane receptors, mobilities, localizations in the peripheral lymphoid organs, and evidently their respective physiological roles. The main objective of this program is to restore the normal function of the system in order to protect immunosuppressed patients against infections, especially of viral origin which are susceptible to be cured by B cell transplantation or transfusion of polyclonal or monoclonal antibodies.

II. Objectives for the reporting period:

The reporting period is devoted to:

- studies of the medium term effects of prenatal or early postnatal irradiations on the immune system of rat;
- development of the rat monoclonal antibodies technology;
- development of rat hybridomas against human leucocytes

III. Progress achieved:

3.1. Studies of the long term effects of prenatal or early postnatal irradiations on the immune system of rats

In collaboration with M. Janowski and R. Hooghe of the Pathology section, Biology Department of the Study Center for Nuclear Energy, B-2400 Mol, Belgium, we have studied for the medium term effects a 0 to 2 Gy, whole body X irradiated rats during approximately two months. The immune responses against T dependent (DNP-OVA) and T-independent (DNP-HES) antigens were both normal or slightly increased in one case for the IgM isotype and only in the case of T-dependent antigens as shown in Table 1. Tissue sections of the spleen, lymph nodes and Peyer patches of animals of the various groups were prepared. We did not observe clear differences between the irradiated rats and their controls (Figure 1).

3.2. Rat monoclonal antibodies against human leucocytes

In collaboration with the immunohematology unit of the Faculty of Medicine of the University of Louvain (Prof. G. Sokal), we have developed a number of rat monoclonal antibodies able to block a non compatible graft rejection. Presently, the most interesting rat monoclonal antibody we have obtained is the LO-Tact-1 which is directed against the human IL-2 receptor. It is able to block alloreactive T cells.
After a special preparation for human use, it has been injected in patients and it appears to block a graft rejection in humans. These preliminary results will be tested during the next year.

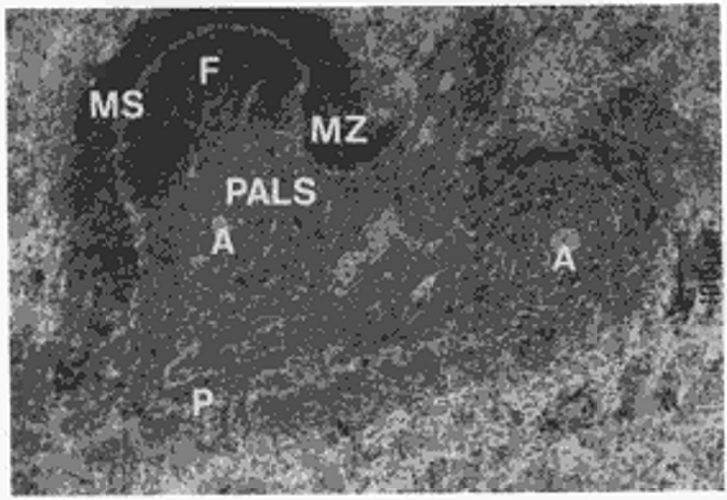

Figure 1: Spleen tissue section of a rat irradiated (whole body X ray irradiation) at 6 days of gestation and sacrified when two month old. MS: marginal sinus; F: follicle; MZ: marginal zone; A: central arteriol; PALS: periarterial lymphocyte sheat; P: plasma cells.

Table 1: Mean values of anti-DNP specific antibodies in DNP-OVA (T-dependent) and DNP-HES (T-independent) immunized rats: sham irradiated rats (A), rats irradiated on a single occasion between days 6 and 12 of gestation (B), between days 14 and 20 of gestation (C) and between days 2 and 8 after birth (D).

Irradiation dose in CGy	Experimental group	Anti-DNP specific antibodies induced by an immunization with					
		DNP-OVA (T-dependent)			DNP-HES (T-independent)		
		N[a]	IgM (µg/ml)	IgG (µg/ml)[d]	N	IgM (µg/ml)	IgG (µg/ml)
0	A	6	107.9[b]	19.3	5	138.7	19.7
25		6	187[c]	23.7	5	144.8	15.6
50	B	4	152.8	23.1	4	109.8	11.8
100		6	124.6	22.7	4	139.9	17.9
50		7	152.2	28.3	7	131.8	16.8
100	C	7	173.9[c]	23.8	7	131.4	17.3
200		4	121	21.8	4	150.7	25.3
50		9	102.8	21.2	9	158.3	15.6
100	D	7	151.2	26.3	7	158.7	15.7
200		8	121.5	24.3	8	191.0	18.9

[a]Number of values corresponding each to a pool of 3-4 sera from animals of the same litter.
[b]Mean
[c]$0.01 > P > 0.05$
[d]IgG1 and IgG2b

IV. Objectives for the next reporting period:

During the next period of the contract, we shall carry on the experiments of pre- or postnatal irradiations of rats in order to determine the values of their immune system: T and B lymphocytes, serum immunoglobulin levels and tests of reactivity of the lymphocyte subpopulations, if possible.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs Michel Janowski and Robert Hooghe, CEN, Department of Radiobiology, Mol, Belgium.

VI. Publications:

1. PUBLICATIONS IN SCIENTIFIC JOURNALS

1.1. Publications not mentioned in the report of 1987

Bazin,H, 1987.Purification of rat monoclonal antibodies.Biochem Life Sci. Adv.,6,135,142

Rits,M, Kints,JP, Bazin,H, Vaerman,JP, 1987.Rat C3 conversion by rat anti-2,4, dinitrophenyl (DNP) hapten IgA immune precipitates.Scand. J. Immunol.,25,359,366

1.2. Publications 1988

Schumcker,DL, Glibert,R, Hradek,GT, Jones,AL, Bazin,H, 1988.Effect of aging on the hepatobioliary transport of dimeric immunoglobulin A in the male Fisher rat.Gastroenterology,88,436,443

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Canon, JL, Humblet, Y, Lebacq-Verheyden, AM, Manouvriez, P, Bazin, H, Rodhain, J, Prignot, J, Symann, M, 1988.Immunodetection of small cell lung cancer metastases in bone marrow using three monoclonal antibodies. Eur. J. Cancer Clin. Oncol.,24,147,150

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Cerny,A, Ramseier,H, Bazin,H, Zinkernagel,RM, 1988. Unimpaired first-set and second-set skin graft rejection in agammaglobulinemic mice. Transplantation,45,1111,1113

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Kints,JP, Manouvriez,P, Bazin,H. Rat monoclonal antibodies. VII.Enhancement of ascites and monoclonal antibody production in rats by pretreatment with Pristane and Freund adjuvant. J. Immunol. Methods.

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2. SHORT COMMUNICATIONS

Regulation of bone marrow (BM) cell differentiation and migration into peripheral lymphoid organs (PLO) by certain bacteria and bacterial cell wall components. 6th International Congress of Immunology, Toronto, Canada, 6-11 July 1986. Book of abstracts 1.11.19. In collaboration with S.M. CHICE, L. TARCSAY, P. DUKOR, H.G. DURKIN.

Rat monoclonal (MC) IgA anti-dinitrophenyl (DNP) antibodies (AB). Partial activation of rat, but not human complement (C) by IgA immune complexes (IC). 6th International Congress of Immunology. Toronto, Canada, 6-11 July 1986. Book of abstracts 1.43.10. In collaboration with M. RITS, J.P. VAERMAN.

The interaction of various IgG's with solubilized Fc receptors of rat basophilic leukemia cells. 6th International Congress of Immunology. Toronto, Canada, 6-11 July 1986. Book of abstracts 2.61.13. In collaboration with M.R. KEPRON, A. FROESE.

Regulation of IgE isotype expression by certain bacteria and bacterial cell wall components. 6th International Congress of Immunology. Toronto, Canada, 6-11 July 1986. Book of abstract 5.36.20. In collaboration with H.G. DURKIN, S.M. CHICE, L. TARCSAY, P. DUKOR.

Automatisation de la purification d'antigènes par immunoaffinité. Proteins Purification Technologies - 2nd European Symposium. September 29-30, 1986. En collaboration avec J.M. MALACHE.

Antibody-dependent cellular cytotoxicity by K cells: Fc receptors of effector cells bind preferentially certain isotypes. Joint Meeting of the Gesellschaft für Immunologie and the Société Française d'Immunologie. Palais de la Musique et des Congrès, Strasbourg, France, 20-22 novembre 1986. En collaboration avec D.M. CHASSOUX, L.G. LINARES & M. STANISLAWSKI.

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Eradication of a hantavirus infection among laboratory rats by application of cesarian section and foster mother technique. Institute of Tropical Medicine - Antwerpen - December 11, 1987. In collaboration with VAN DER GROEN G., BEELAERT G., HOOFD G., KINTS J.P., F. CORMONT, NISOL F., H. BAZIN.

Rat monoclonal antibodies against neural crest derived cancers. Possible clinical applications. Belgian Hematological Society. Third General Meeting. Brussels, 23.1.1988. A.M. RAVOET, J. NINANE, A. NEIRYNCK, Y. HUMBLET, M. SYMANN, G. SOKAL.

Mouse immunoglobulin isotype determination with rat-rat monoclonal antibodies. Joint BIS and monoclonal antibodies study group meeting. Institute of Molecular Biology, Rhode St. Genèse - February 19, 1988. In collaboration with P. MANOUVRIEZ, T. DELAUNAY.

Rat IgG2b monoclonal antibodies directed against human interleukin-2-receptor potentially useful for the reversion of allograft rejection. Réunion Commune de la Société Française d'Immunologie et de la Société Anglaise d'Immunologie. PARIS, Centre de Congrès de la Vilette, Avril 7-8 1988. En collaboration avec A.M. RAVOET, D. LATINNE, P. MANOUVRIEZ, H. BAZIN, J. NINANE, M. DE BRUYERE, G. SOKAL.

IgE dependent cellular cytotoxicity. Réunion Commune de la Société Française d'Immunologie et de la Société Anglaise d'Immunologie. PARIS, Centre de Congrès de la Vilette, Avril 7-8, 1988. In collaboration with D.M. CHASSOUX.

Immunity and aging. XIXth International Congress of Internal Medicine. Brussels, 29th August-2nd September 1988. H. BAZIN.

Purification of mouse monoclonal antibodies by immunoaffinity chromatography. European Society for Animal Cell Technology. The 9th Meeting "Advances in Animal Cell Biology and Technology for Bioprocesses" Knokke (Belgium) - September 26-30, 1988. H. BAZIN.

EC programmes in biotechnology: BAP, BRIDGE, ECLAIR, FLAIR - An overiew. European Society for Animal Cell Technology - The 9th Meeting "Advances in Animal Cell Biology and Technology for Bioprocesses" Knokke (Belgium) - September 26-30, 1988. H. BAZIN.

Possible existence of common antigens between Streptococcus mutans cell wall and human heart and kidney tissues. Société Française d'Immunologie and British Society for Immunology, Paris, avril 1988. In collaboration with F. ACKERMANS, J.P. KLEIN.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-057-UK

Medical College of
St. Bartholomew's Hospital
West Smithfield
GB - London EC1A 7BE

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.E. Coggle
Department of Radiation Biology
Med. Coll. St. Bartholomew's Hosp.
Charterhouse Square
GB - London EC1M 6BQ

Telephone number: 01-251 1184

Title of the research contract:

Stochastic and non-stochastic effects of alpha and beta radiation on mouse skin.

List of projects:

1. Non-stochastic effects of alpha and beta radiation on mouse skin.
2. Stochastic effects of alpha and beta radiation on mouse skin.

Title of the project no.: 1

Non-Stochastic Effects of Alpha and Beta Radiation on Mouse Skin.

Head(s) of project: Dr. J.E.Coggle

Scientific staff: Mrs. S.G.Needham

I. Objectives of the project:

To answer the following practical radiation protection questions: (1) Is there a minimum area that needs to be irradiated to induce moist desquamation and ulceration? (2) What are the threshold doses for these two effects and how do they vary as a function of area irradiated? (3) How do the thresholds vary with radiation energy? (4) What are the RBE values for alpha particles for these non-stochastic effects? (5) How do the dose response curves for desquamation and ulceration vary with the protraction of alpha and beta doses?

II. Objectives for the reporting period:

(1) To produce a series of dose response curves for acute skin reactions following a range of curium-244 alpha doses for a variety of source sizes.

(2) To complete the DNA labelling and vincristine cell birth rate studies on basal cells to determine the relative roles of surviving follicle cells and field-edge cells in the regenerative response of skin.

(3) To investigate the anomalous MD-50 and threshold data for the small sources and in the light of detailed analysis of all the non-stochastic results, establish accurate macroscopic criteria for the various energy emitters.

III. Progress achieved:

Methodology.

A. Moist Desquamation Experiments

The details for experimental procedure used to assess both the moist desquamative and ulcerative reactions of mouse skin were given in the 1986 annual report. We continued to use the same procedure to assess the acute response of mouse skin to a variety of beta sources and also to a range of curium-244 alpha doses. To date, the latter has been with a single source of 8 cm^2 area.

Results

Work this year on beta sources involved a more critical analysis of the acute responses observed to date, with particular reference to the thulium and strontium sources. It was found that the severity of the acute observations could be divided into three distinct levels of reaction which had temporal, as well as subjective intensity, criteria. These could be described as follows: an early, low level, dry desquamative reaction; a synchronised, short duration, moist desquamation; a much more severe, prolonged ulcerative response. The observed temporal aspects of the reactions showed an area effect in addition to a dose related response This analysis is now being extended to all of the beta sources.

The acute reaction studies for the 8 cm^2 curium-244 source has now been completed. At the highest dose level used (180 Gy), none of the animals exhibited moist desquamation. The acute response began as a mild erythema on day 2 post-irradiation, reached a peak response on day 6, when some 66% of the mice exhibited a number of dry flakes. Flaking was observed until approximately day 10, thereafter only mild erythema was seen persisting in only a few animals for two or three days. At lower dose levels (120 and 80 Gy), there was a dose related increase in the time to reaction onset and a dose related reduction in the reaction duration, although the peak in response still appeared on days 6 and 7 respectively.

At dose levels below 80 Gy, the only discernible gross macroscopic reaction was a small degree of "mottling" in the skin coloration. At all dose levels, the acute reaction became difficult to score beyond 12 days post-irradiation since there was no disruption to the hair growth cycle beyond a slight delay, so that at this time the hair regrowth obscured the irradiated field. The histopathology on these reactions is yet to be

completed.

Once again, one of the objectives of this period was to investigate the anomalous MD-50 values given by the 0.8 mm^2 strontium-90 and thulium-170 sources. However, we are still awaiting the detailed dosimetry data of these two sources from our collaborating laboratory.

The final objective for this period was to complete the study of the DNA labelling indices of mouse skin to establish the relative roles played by follicle and field-edge epithelial cells in epidermal regeneration. Some difficulties have been experienced and these studies are not yet completed.

IV. Objectives for the next reporting period:

(1) To complete the observations for acute skin reactions following curium-244 alpha doses using a variety of sources sizes / uniformity.

(2) To complete the DNA labelling and vincristine cell birth rate studies on basal cells to determine the relative roles of surviving follicle cells and field-edge cells in the regenerative response.

(3) To produce a series of acute dose response curves for moist desquamation for the four strains of mice involved in the stochastic experiment, carrying out detailed histology with respect to their normal morphology in addition to their acute reactions.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. M.W.Charles and Dr. J.Wells, Health Physics Research, Berkeley Nuclear Laboratories, Central Electricity Generating Board, Berkeley, Gloucestershire GL13 9PB.

Dr. J.W.Hopewell and colleagues, CRC Normal Tissue Radiobiology Research Group, Churchill Hospital, Headington, Oxford OX3 7LJ.

VI. Publications:

None in this period of the contract.

Title of the project no.: 2

Stochastic Effects of Alpha and Beta Radiation on Mouse Skin

Head(s) of project: Dr. J.E.Coggle

Scientific staff: Mrs. S.G.Needham

I. Objectives of the project:

Human exposure to radiation is rarely uniform over a tissue and most of the data used in cancer risk assessment are derived from non-uniform, partial body exposure. Therefore it is important for radiation protection to design model experiments involving comparisons of uniform and non-uniform radiation exposure. The aim of this project is to develop dose response curves for skin cancer in mice given uniform and non-uniform alpha and beta irradaition and so test the ICRP assumption that for a given average tissue dose, inhomogeneous irradiation of a small area of skin is less carcinogenic than the same dose given uniformly over a larger area.

II. Objectives for the reporting period:

(1) In the uniform / non-uniform project, continue monitoring the mice for tumours/morbidity. Continue the pathological studies of the induced tumours to qualify the target cells in both the epidermis and dermis.

(2) Continue to monitor the four strains of mice for cancer proneness.

(3) Complete the exposure programme for the different dose rate thulium-170 sources. Continue monitoring the mice for tumours/ morbidity.

(4) Commence the skin cancer induction project using curium-244 alpha sources.

III. Progress achieved:

Methodology.

A. Uniform, Thulium-170 Dose Rate Experiments.

The 1987 annual report gave the essential details of the experimental procedures used to irradiate and monitor mice exposed to large area uniform thulium sources.

B. Skin Cancer Induction Following Curium-244 Alpha Irradiation.

The experimental procedure for the irradiation of mouse skin using uniform curium-244 alpha sources is essentially the same as described for the uniform thulium-170 beta source in the 1987 annual report. The curium-244 sources have an activity of 100 uCi distributed uniformly over an area of 8 cm^2. The sources are sealed, but have an additional 4 um melinex wrapping and the mice are irradiated through a supporting film of 6 um melinex. The dosimetry of the sources has shown a dose rate at the mouse skin surface of 260 Gy per hour. At depths of 10, 20 and 25 um in mouse skin, the alpha doses are 179, 89 and 49 Gy per hour respectively; a depth of 30 um in mouse skin is beyond alpha particle penetration range.

Results.

A. Uniform / Non-Uniform Thulium-170 Experiments.

None of the 152 control SAS/4 mice developed tumours within the the experimental area.

This experiment is now completed. Tumours were found in all dose groups for the uniform and both 32- and 8-array non-uniform sources. There was a suggestion of a dose related minimum latent period, although this was not as long as previous work from this laboratory had indicated. The final cumulative tumour incidences for the 120 - 129 week period were: for the uniform source 2, 5, 10 and 20 Gy groups, 11.1%, 2.6%, 4.6% and 36.7% respectively; for the 32-array 2 Gy group, the cumulative tumour incidence was 11.3%; finally, for the 8-array 2 and 10 Gy groups, the cumulative tumour incidences were 2.7 and 19.9% respectively.

Many of these final incidences were disproportionately high through the late appearance of tumours in the small surviving groups; the three sources actually induced relatively equal numbers of tumours, the time of tumour development affecting the overall appearance of the incidence curves.

The data from this experiment were combined with results from work done earlier in this laboratory and the essential linearity of the early

part of the induction curve was confirmed. A linear regression analysis of the data over the range 0 D 100 Gy showed a straight line fitted the data, constrained to pass through the origin by the method of least squares.

The pathological studies so far have shown that in the SAS/4 mouse strain, following beta irradiation, 6% of the tumours induced are of epidermal origin, the remainder being of either dermal or other origin. Of the epidermal tumours, squamous cell carcinomas appear within the first 30 - 70 weeks post-irradiation; any epidermal tumours arising later were either squamous cell papillomas or of follicular origin. The majority of dermal tumours have been classified pathologically as rhabdomyosarcomas, although further histological work is being carried out to confirm this diagnosis.

B. Study into Strain Differences with respect to Tumour Induction.

These are life time experiments and the majority of the groups at present (approximately 100 weeks post-irradiation) still have many surviving members. However, the preliminary results are proving very interesting, although pathological confirmation of tumours must still be made in many cases.

Two albino strains, CD1 and SAS/4, were irradiated over a dose range of 12.5 - 100 Gy. At the 90 - 99 week post-irradiation period, the CD1 strain shows a 16% cumulative tumour incidence in the control group. This predisposition towards skin tumours appears to have been enhanced by the uniform irradiation; preliminary estimates show an approximate 40% cumulative tumour incidence at 12.5 Gy for this period and 60% incidence for the higher dose levels. There is a strong dose related response, although there is an indication of the top three dose groups starting to plateau at the 90 - 99 week point. The tumours start to appear in the control group during the 70 - 79 week period with a corresponding acceleration in tumour induction in the dose groups.

To date, none of the SAS/4 control group has developed a tumour in the experimental area. The 12.5 Gy group shows a similar pattern of incidence to that seen in the low dose experiment above; the first tumour arose in the 60 - 70 week period and the cumulative tumour incidence has appeared to plateau at about 5%. The two higher groups of 25 and 50 Gy had shorter minimum latent periods, but have also plateaued at 16% and 17% tumour incidence respectively. The 100 Gy group has shown a steady

increase in cumulative tumour incidence to its present level at the 90 - 99 week period of 39%. All results are preliminary pending pathological verification.

The C57BL/6 strain has shown a mixed response, although as with both of the above strains, tumours have been induced at all dose levels. No tumours have been seen in the control group. Until the 70 - 79 week period, the C57BL/6 strain showed a similar pattern and level of response to that of the SAS/4 strain. However, since that point, the two intermediate dose groups of 25 and 50 Gy show no plateau in their cumulative tumour incidence, but an increase to 28% and 34% respectively, whilst the 100 Gy group shows a reduced cumulative tumour incidence of 22% in comparison with that of the 100 Gy SAS/4 group's tumour incidence of 39%.

The CBA strain have produced a single possible tumour in the experimental area in the control group, but no tumours have as yet been seen in the 12.5 Gy group, although tumours have been found at the three highest doses. This strain appears to show a much increased minimum latent period compared with the other three strains, although this may be a reflection of its longer life span and analysis of this may throw an interesting light on the problems of extrapolating data from the short-lived mouse to the much longer-lived man.

The pathology and histogenesis of the induced tumours should prove interesting in the light of questions concerning target cells.

C. With respect to the dose rate experiment, the low dose rate group has shown a longer minimum latent period than the high dose rate group and the cumulative tumour incidence has been reduced by one third. However, results are still too preliminary for statistical analysis.

D. In the curium-244 alpha experiments, no skin tumours were expected and none have been recorded so far.

IV. Objectives for the next reporting period:

(a) Continue to monitor the four strains of mice for tumours / morbidity. Extend the studies of the pathology of the induced tumours.

(b) In the dose rate project, continue to monitor the mice for tumours / morbidity.

(c) Complete the exposure programme for the curium-244 alpha sources. Continue to monitor the mice for tumours / morbidity.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. M.W.Charles and Dr. J.Wells, Health Physics Research, Berkeley Nuclear Laboratories, Central Electricity Generating Board, Berkeley, Gloucestershire GL13 9PB.

Dr. J.W.Hopewell and colleagues, CRC Normal Tissue Radiobiology Research Group, Churchill Hospital, Headington, Oxford OX3 7LJ.

VI. Publications:

Charles M.W., Williams J.P., Coggle J.E. Skin carcinogenesis following uniform and nonuniform beta irradiation. Health Physics 55 (2), 399-406, 1988.

Williams J.P. Skin carcinogenesis in the mouse following uniform and non-uniform beta irradiation. Ph.D. Thesis, University of London, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Commissariat à l'Energie
Atomique, CEA
B.P. n° 510
rue de la Fédération, 39
F - 75752 Paris Cédex

Contract no.: BI6-C-058-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Daburon
Laboratoire de Radiobiol. Appliquée
CEA-IPSN
F - 78350 Jouy-en-Josas

Telephone number: 3-956.29.15

Title of the research contract:

Problems related to skin and underlying tissues after accidents involving local irradiation. Experimental study in the pig.

List of projects:

1. Problems related to skin and underlying tissues after accidents involving local irradiation. Experimental study in the pig.

Title of the project no.:

Problems related to skin and underlying tissues after accidents involving local irradiadiation. Experimental study in the pig.

Head(s) of project:

Dr F. DABURON

Scientific staff:

Drs D.HOFFSCHIR, J.L. LEFAIX, M. MARTIN and J. REMY

I. Objectives of the project:

Diagnosis, prognosis and treatment of acute localized irradiated pigs and rabbits. Attempts to establish dose-effect relationships and to evaluate the size and intensity of the radiolesions, as an aid for operating, by non invasive methods.

Pathogenic studies of early and late effects in irradiated tissues by histological, histochemical, immunological and cellular culture methods.

Molecular studies of matrix synthesis by post-irradiation fibrosis extracted fibroblasts.

II. Objectives for the reporting period:

- Therapeutic trials in locally irradiated rabbits. Clinical, pathological and histological studies.

-Evaluation of 201 Tl uptake as an aid for the characterization of early and late muscular radiolesions in pigs.

-Early surgical treatment of acute localized irradiation in pigs.

-Post irradiation fibrosis studies: molecular studies of matrix synthesis; attempts of modulation of fibrosis fibroblasts by different cytokines.

III. Progress achieved:

1. Methodology

-Acute localized irradiations were performed with a 192 Ir collimated source:

-on the left part of the back (m. iliospinalis) of rabbits at 20 Gy.

-on the right thigh (m. biceps femoris) of pigs at 30 and 60 Gy.

Doses are given at 2 cm depth; at skin basal layer level the dose is 4 times higher.

-Rabbits were observed during 40 weeks according to 8 groups of 21 animals, respectively as control (1) and treated (7) by corticoïd, non-steroïdal anti-inflammatory, vasodilatator, anti-ischemic or platelet anti-aggregant drugs.

-Pigs (3 at 30 Gy and 3 at 60 Gy) were studied from 2 to 4 months after irradiation by 201 Tl gamma scintigraphy; 133 Xe scintigraphies were performed after indwelling catheterization of art. renalis and aorta abdominalis to avoid the first lung passage which decreases the blood burden by 90 %.

-Six 60 Gy irradiated pigs were early operated (1st to 3rd day): only irradiated skin was removed.

-Skin, muscles and fibrotic tissues were removed from pigs from 6 to 20 months after irradiation. All these tissues and cell lines derived from fibrosis and newborn skin were investigated for the mRNAs content of major matrix proteins. Adult pig lymphocytes were used as control. 32 P labelled cDNA probes (pro alpha 1(I) collagen, pro alpha 1(III) collagen and alpha actin) were hybridized with total extracted RNA using blotting technique.

Results

-Diagnosis: scintigraphy in pigs

Four months after irradiation an hyperfixation (4 times the control level) was observed in the muscular radiolesions and involved all the fibrous tissue, as it was shown by comparison between scintigraphy and anatomical observation at slaughter.On the other hand analysis of 133 Xe scintigraphies showed the progressive development of an hypervascularization concomitant of the ulcer formation and the increase of 201 Tl uptake.

-Treatment.

Surgery in pigs: early skin excision weakened the early inflammatory response and limited the fibrosis extension into the muscular tissue irradiated at doses higher than 30 Gy.

Pharmocology in rabbits: the efficience of the treatments was estimated by delaying the clinical evolution of the lesions, decreasing the early inflammatory reaction and weakening the intensity of the subcutaneous lesions. Betamethasone and the combination of flurbiprofene and trimetazidine were the most effective treatments, when vasodilatators (naftidrofuryl or buflomedil) and platelets anti-agregant (pyricarbate) were almost ineffective.

Molecular biology of fibrosis -tissues: the collagen mRNAs levels decrease according to the following order: skin, fibrosis, newborn skin,muscle. Although the content of collagen was smaller in fibrosis than in skin, the ratio of type I to type III mRNA was smaller in fibrosis.Data on newborn skin were similar to those on fibrosis.

-cells: fibrosis cells exhibited a higher level of actin and fibronectin mRNA; they synthesized more collagen III mRNA than skin cells, as illustrated by a smaller type I to type III ratio. However skin cells synthesized more collagen I mRNA.

Discussion

By comparison between histological and scintigraphic results, 201 Tl seems to have an important affinity for highly vascularized tissues with a high content of fibroblastic and inflammatory cells: in the development of the radiolesions it would be a first marker of inflammatory processes and later of fibrotic processes.

Early removal of irradiated skin in pigs involves two unexpected beneficial effects by weakening the early inflammatory reaction and avoiding solution of continuity in the cutaneous integument: persisting injuried skin promotes deep infection and necrosis extension in the underlying muscular tissue, even irradiated below 30 Gy.

Treatments in rabbits act like decreasing the dose, as it was already seen in pigs; few differences were noted in rabbits concerning the pharmacological effects (except for antithrombosis drugs).

As a conclusion on molecular studies, we assume that the fibrosis cells increase their matrix synthesis by enhancing the DNA transcription processes, mainly for fibronectin and collagen III genes. However, other steps of regulation during post-transcriptional events are probably involved in abnormal matrix deposition, especially in collagen I synthesis.

IV. Objectives for the next reporting period:

-Studies of cell populations involved in hyperfixation of 201 Tl by inflammatory or fibrous tissues of the pig.

-Starting of electromyographic studies in irradiated skeletal pig muscle.

-Carrying on pharmacological studies in irradiated rabbits.

-Development of collagen studies by new molecular techniques: in vitro transcription measurements of mRNA stability.

-In vitro modulation of fibrosis extracted cells by growth factors: TGF β or IFN γ.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

-Laboratoire Central de Pathologie, Hôpital Saint-Louis, 2 Place du Docteur Fournier, 75010 Paris (Prof. Brocheriou).

-Laboratoire de Biochimie du Tissu Conjonctif G.R. C.N.R.S. 40, Institut Universitaire de Recherche sur les Maladies Vasculaires. Faculté de Médecine, 8 rue du Gal Sarrail, 94010 Créteil (Prof. Robert).

VI. Publications:

1. Lefaix J.L., Daburon F., Martin J.L., Jeandey C.-Interêt de l'analyse en composantes principales appliquée à l'imagerie RMN pour le diagnostic précoce de l'irradiation aiguë localisée, étude expérimentale chez le lapin. I.T.B.M., 1988, vol. 9, N° 4, 429-445

-Wegrowski J., Lafuma C., Lefaix J.L., Daburon F., Robert L.- Modification of collagen and noncollagenous proteins in radiation-induced muscular fibrosis. Experimental and Molecular Pathology, 1988, 48, 273-285

2.El Nabout R., Martin M., Remy J., Robert L., Lafuma C.-Collagen synthesis and deposition in cultured fibroblasts from subcutaneous radiation-induced fibrosis. Modification in function of cell ageing. XIth FECTS Meeting, Amsterdam, 1988, Abs. N° 176

-El Nabout R., Martin M., Remy J., Petitou M., Choay J., Robert L., Lafuma C. Heparin fragments modulate the collagen phenotype of fibroblasts from radiation induced subcutaneous fibrosis. XIth FECTS Meeting,Amsterdam, 1988, Abs. N° 9

-Martin M., El Nabout R., Remy J., Lafuma C., Daburon F.-Ageing and collagen synthesis in normal and fibrotic pig fibroblasts in culture. XIth FECTS Meeting, Amsterdam, 1988, Abs N° 178

-Remy J., Wegrowski J., Créchet F., Martin M., Daburon F.- Collagen in post-irradiation fibrosis; biochemical and molecular studies. XIth FECTS Meeting, Amsterdam, 1988, Abs. N° 22

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-C-176-IRL

The Health Research Board
73 Lower Baggot Street
IRL - Dublin 2

Head(s) of research team(s) [name(s) and address(es)]:

Dr. G. Dean
c/o The Health Research Board
73 Lower Baggot Street
IRL - Dublin 2

Telephone number: 1-761176

Title of the research contract:

Superficial radiotherapy to the eye and the risk of developing cataract.

List of projects:

Superficial radiotherapy to the eye and the risk of developing cataract.

Title of the project no.: Superficial radiotherapy to the eye and the risk of developing cataract.

Head(s) of project: Dr. Geoffrey Dean, MD, FRCP.(I), FFCM.(I).

Scientific staff: Professor Michael Marmot, Dept. of Community Medicine, University College,London. (Collaborating with study).

Scientific Advisers. Dr.Ebert and Dr. Gerber. EEC. Brussels.
Dr. R.Maximilien, Euratom,Fontenay-aux-Roses.

I. Objectives of the project:

To obtain the subsequent history of patients treated with radiotherapy for inflammatory conditions of the eye between 1950 and 1970 and of patients who had alternative eye therapy. The general practitioners of those who are alive were asked to provide a subsequent history of the patients, whether they had developed cataract and whether the cataract had been removed. A comparison has been made between those who had radiotherapy and those who had alternative treatment.

II. Objectives for the reporting period:

The reports on the patients were obtained from the patients' doctors, the information was analysed and a report for the Commission and for publication was prepared.

1. METHODOLOGY

The present study compares patients who have received superficial radiotherapy to the eye for various inflammatory disorders with patients who have received alternative therapy for similar eye conditions. This is to ascertain whether it is the eye condition which was responsible for the risk of cataract or whether it was the radiotherapy and also to ascertain if the risk of cataract was radiation dose related.

Details of patients were obtained from six hospitals where radiation therapy had been given for a variety of eye conditions, in particular for inflammatory eye disorders and keratitis. This was usually X-ray therapy but a few patients were treated by strontium plaque. Similar information about the patients was obtained from a seventh hospital which did not give radiation therapy for similar eye conditions but used alternative local treatment, anti-infectious and anti-viral agents, cortisone, etc.

Cards were completed for each patient describing the treatment and noting the name, address and, where known, date of birth or estimated age. These cards, without the medical details, were then sent to the National Health Service (NHS) Central Register, Southport, where a search was made to find the names of the patients'Family Practitioner Committees(FPCs) The NHS Central Register holds the name of the patient's FPC but not the name of the patient's doctor. For the patients who were alive and traced and registered with a FPC, the Administrator of the FPC was asked to forward to the patient's doctor a letter explaining the research and asking the doctor to return in a stamped addressed envelope a completed form describing the present condition of the patient. This form provided the name, forename, date of birth, the NHS number, the type of the cataract and whether it was anterior or posterior, the date of cataract diagnosis, whether seen by an ophthalmologist, whether the lens was removed from the right, left or both eyes and the date of the operation.

2. RESULTS

Records were obtained for 427 patients from the hospitals who had received superficial radiation therapy for eye disorders, excluding neoplasm. Of these 258 had died or could not be traced by the NHS Central Register and 169 were traced to the FPCs and were thought to be alive. For patients who had received treatment other than radiation therapy, 565 records were obtained of whom 323 had died or could not be traced and 242 were traced to their present FPC. TABLE 1. Excluding those who had died since the start of the study, those who could not be traced by the FPC, those who were unknown to the doctor and those for whom the doctor did not reply, there were 144 responses from the doctors for patients who had radiation therapy and 194 among those who had alternative therapy.

On analysis of the 144 responses for patients who had received radiation therapy, 69 males, 75 females, 28 had developed cataract, 13 of these had had one and 3 had had two cataract operations. Twenty-two had other eye pathology which made it impossible to determine whether they had cataract or not and 94 had no eye pathology at the time of reporting. Of the 194 responses for the patients who had other therapy, 103 males, 91 females, 9 had developed cataract 8 had other pathology which made it impossible to examine the lens and for 177, 98 males, 79 females, no eye pathology was reported.

The age distribution of the patients in 1988 is shown in TABLE 2 and also the number that developed cataract or had other eye pathology both for those who had radiation therapy and those who had alternative therapy in each age group. For the radiation therapy group, the mean ages of the 28 patients having cataract were: males 76.4 years and females 77 years. The mean ages of the 94 persons not having any recent eye pathology were: males 59.8 years and females 67 years. For the patients who had other therapy, the mean ages of those having cataract (9) were: males 40 years and females 71.7 years. For the 177 persons not having any eye pathology, the mean ages were: males 48.7 years and females 50.5 years.

For those who had received radiation therapy, the expected number to have developed cataract, or have other eye pathology, if they had had the same risk as those who had no radiation therapy, has also been calculated. This takes account of the differences in age distribution of the two populations. Among the 144 patients who received radiation therapy, the "expected" number to have developed cataract, at the rates occurring among those who had no radiation therapy, was 17.1 and the actual number was 28, a significant difference, ($P < 0.01$). Below the age of 80 years, 18 of the patients who received radiation therapy developed cataract and the expected number was 8.7, a highly significant difference ($P < 0.001$). Twenty-two had other eye pathology, some of whom may have had cataract but the lens could not be examined and the expected number was 6 ($P < 0.001$). Among those who received radiation therapy there were 50 patients out of 144 at risk who had cataract or other eye pathology; the expected number was 23.1. Nine of the 22 with other eye pathology had been treated by strontium plaque.

The commonest conditions that were treated among both groups of patients were corneal ulcerations, corneal vascularisation, keratitis, corneal abrasions, pterygium or iridocyclitis. The characteristics of the patients who developed cataract are shown in TABLE 3. The mean number of years between radiation therapy and operation was 8.2. The type of cataract was reported in six cases, four anterior and two posterior.

Among the 144 patients who received radiation therapy 22 had developed eye pathology which made it difficult

or impossible to determine whether or not they had cataract. In 16 thi was corneal scarring, in four the eye had been enucleated, one patient was reported as "blind" and one suffered from severe glaucoma. Of the 8 out of the 194 who had received alternative therapy and who had other eye pathology, 5 had corneal scarring, 1 had had the eye enucleated and in 2 there was severe glaucoma.

Seven patients out of 57 treated by 10 Gy or less superficial X-ray therapy developed cataract and the expected number calculated for those receiving non-radiation therapy was 7.3, no significant difference. The increased risk of cataract occurred in those who received more than 10 Gy radiation; there was no difference between those who had received 11-20 Gy radiation (11 actual and 4.8 expected) and those who had received 20+ Gy (10 actual and 5 expected). TABLE 4. The patients who received more than 10 gy radiation therapy had a significantly greater risk of developing cataract, 21 out of 87 patients, than the expected number, calculated from those receiving alternative therapy, 9.8. ($P < 0.001$).

Other eye pathology occurred significantly more frequently both among the patients who had had 10 Gy or less, 8 and 2.5 expected, and among those who received more than 10 Gy, 14 and 3.5 expected, at the rate in patients who did not have radiotherapy. Two of the eight patients who had 10 Gy or less and seven of the patients who had had more than 10 Gy and had other eye pathology had been treated by strontium plaque.

Recently, Ron et al. have confirmed that radiation dosage of the order of 1 to 2 Gy can significantly increase the risk of neural tube defect if given in childhood. It would be interesting to see if children also have a bigger risk of developing cataract from radiation than occurs in adults.

The Hospital Activity Analysis (HAA) Reporting System reports each year the number of operations for cataract by sex and age group. Based on this information, the calculated lifetime risk of having had a cataract operation for the population in this study which had radiation was by 1988 4.6 and the actual number of operations was 19 (in 16 people), a highly significant difference. For those who have had alternative eye therapy, a younger population with fewer very old people, the lifetime risk by 1988 was 2.2 and the actual number to have had a cataract removed was 6. Although those who had radiotherapy had a greater risk of developing cataract than those who had alternative treatment, table 2, those who did not have radiotherapy had a greater chance of having a cataract removed than would be expected from the general population of England and Wales, although the numbers are small. This suggests that either the original eye condition or the treatment, or both, increased the risk of cataract. (Morbidity Surveillance,England and Wales, 1968-78. Cause).

DISCUSSION

This study confirms the previous study (Dean et al,1988) that there is an increased risk of cataract among those receiving radiotherapy for eye conditions. In the present study, patients who received more than 10 Gy superficial X-ray therapy for eye disorders had a highly significant increased risk of developing cataract. Among those receiving 10 Gy or less, no increased risk of developing cataract above those having alternative treatment could be detected. This is evidence that the risk of cataract is dose dependent. Merriam and Focht have estimated that a radiation dose of 7.5 - 9.5 Gy gave a 50% chance of developing cataract but in this study 10 Gy or less of superficial radiotherapy did not show any increased risk of producing cataract. Perhaps this difference is because the patients in this study were given superficial "non-penetrating" radiation.

More of the patients who had received radiotherapy have to-day pathology other than cataract than those who received alternative therapy, both among those who had 10 Gy or less and among those who had greater than 10 Gy radiation dose. Nine of the 23 patients in this group had been treated by strontium plaque. It may be that the original pathology was more severe among those treated by strontium plaque and they were more likely to be left with permanent corneal damage.

Patients who had received treatment other than radiation had a greater risk of having an operation for cataract than occurred in the general population but not as high a risk as occurred in those receiving radiotherapy.

In conclusion, this study is evidence that radiotherapy for non-malignant eye disorders should, if possible, be avoided because of the increased risk of developing cataract and other eye pathology later in life.

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Office of Population Censuses and Surveys, 1981. Morbidity Surveillance, England and Wales, 1968-78. Cause. HMSO, London.

Ron E, Modan R, Boice JD, Alfandary E, Stovali M, Chetrit A and Katz L. Tumors of the brain and nervous system after radiotherapy in childhood. 1988. New England Journal of Medicine. 319, 16, 1033-39.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None

V. Publications:

Dean G, Alderson M, Maximilien R. Increased risk of cataract in patients receiving radiotherapy to the eye: a pilot study. 1988. Brit. J. Rad. 61, 309-311.

TABLE 1

Results from tracing the patients

Tracing Patients	(a) Patients having radiotherapy to eyes	(b) Patients having treatment other than radiotherapy
Hospital record of treatment	427	565
Untraced at NHS Central Register or died	258	323
Traced	169	242
Response		
Died since start of study	1	5
Not traced by FPC	3	6
Traced by FPC but unknown to doctor	6	7
No reply from doctor	15	30
Total negative response	25	48
Total positive response	144	194
Eye pathology identified by doctor		
Cataract	28	9
Other eye pathology	22	8
No eye pathology	94	177

TABLE 2

Comparison of patients receiving no radiotherapy and radiotherapy to the eye

Age 1988	No radiotherapy: No. of persons 1988	No pathology	Cataract	Other eye pathology	Radiotherapy: No. of persons 1988	No pathology	Cataract	Other eye pathology	Expected number at no radiotherapy rates: Cataract	Other eye pathology
< 50	102	98	1	3	19	14	-	5	0.4	0.6
50-59	32	28	1	3	43	16	1	6	0.7	2.4
60-69	35	33	2	-	47	33	8	6	2.7	-
70-79	20	15	3	2	34	21	9	4	5.1	3.4
80+	5	3	2	-	21	10	10	1	8.4	-
Total	154	177	9	8	144	94	28	22	17.1	6.0
Males	105	100	1	4	69	45	10	14		
Females	89	77	8	4	75	49	18	8		
Cataract operation			6				16			

Radiotherapy (1) Cataract. Actual 28, expected at "no radiotherapy" rates 17.1 χ^2 = 6.9, P<0.01
(2) Other eye pathology. Actual 22, expected at "noradiotherapy" rates 6.0 χ^2=42.7, P < 0.0001.

TABLE 3

Characteristics of 28 patients who received radiotherapy for eye conditions and were reported as having cataract.

Age at treatment

< 20	-
20-39	4
40-49	6
50-59	13
60-69	4
70-79	1

Year of treatment

Before 1950	1
1950-1959	9
1960-1970 incl.	18

Age in 1988

50-59	1
60-69	8
70-79	9
80+	10

Age at diagnosis (6 not known)

< 20	1
20-39	-
40-49	3
50-59	8
60-69	3
70-79	4
80+	3

Age at operation (2 not known)

30-39	1
40-49	1
50-59	7
60-69	2
70-79	2
80+	1

Three patients had a second cataract operation = 19 operations

Characteristics of 9 patients who received alternative treatment for eye conditions and were reported as having cataract.

Age at treatment

< 20	1
20-39	3
40-49	2
50-59	3
60-69	-
70-79	-

Year of treatment

Before 1950	-
1950-1959	1
1960-1970 inc.	8

Age in 1988

40-49	1
50-59	1
60-69	2
70-79	3
80+	2

Age at diagnosis (2 not known)

40-49	1
50-59	1
60-69	1
70-79	4

Age at operation

50-59	1
60-69	2
70-79	3

None had a second cataract operation.

TABLE 4

The effect of radiation dose

The expected number based on those not receiving radiation in brackets.

Age 1988	10 Gy or less: No. of persons	No pathology	Cataract	Other eye pathology	More than 10 Gy.: No. of persons	No pathology	Cataract	Other eye pathology
50	9	6	- (0.1)	3* (0.3)	10	8	- (0.1)	2** (0.3)
50-59	10	7	1 (0.3)	2 (0.9)	13	9	- (0.4)	4* (1.2)
60-69	14	11	2 (0.8)	1 -	33	22	6 (1.9)	5* -
70-79	14	12	1 (2.1)	1* (1.4)	20	9	8 (3.0)	3*** (2.0)
80+	10	6	3 (4.0)	1 -	11	4	7 (4.4)	- -
Total	57	42	7 (7.3)	8 (2.6)	87	52	21 (9.8)	14 (3.5)

* = Patient had been treated by strontium plaque.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-059-I

Com. Naz. per la Ricerca e lo
Sviluppo dell'Energia Nucleare e
delle Energie Alternative, ENEA
Viale Regina Margherita, 125
I - 00198 Roma

Head(s) of research team(s) [name(s) and address(es)]:

Prof. G. Doria
Laboratorio di Patologia
ENEA-CRE Casaccia
C.P. 2400
I - 00100 Roma A.D.

Telephone number: 06-30483619

Title of the research contract:

Radiation damage and recovery of the immune system.

List of projects:

1. Age-related changes in immunological functions in relation to radiation exposure.

Title of the project no.:

Age-related changes in immunological functions in relation to radiation exposure.

Head(s) of project:
Prof. Gino Doria
Laboratorio di Patologia, ENEA C.R.E. Casaccia
C.P. 2400, I-00100 Roma A.D.

Scientific staff:

Dr. L. Adorini
Dr. D. Frasca

I. Objectives of the project:

1. Recovery of T cell functions in irradiated mice by immunoregulatory molecules.

2. Characterization of the interaction between the T cell receptor and antigenic epitopes associated with MHC class II molecules.

II. Objectives for the reporting period:

1. Restoration of helper T cell activity and IL-2 production in irradiated and old mice by a synthetic nonapeptide of human IL-1 β

2. Mapping of antigenic epitopes that interact with MHC class II molecules and the T cell receptor.

III. Progress achieved:

The immune response to exogenous and endogenous antigens is induced by the activation of antigen-presenting cells (macrophages, dendritic cells, and B cells) and modulated by T cells and factors which regulate the intensity and duration of the response. Interleukins play a major role in the amplification of immune responses. Hence, antigen-stimulated macrophages synthesize and release interleukin 1 (IL-1), which induces the production and expression of interleukin 2 (IL-2) and IL-2 receptors in activated T cells and promotes B cell growth and differentiation, as well as antibody secretion . IL-2, in turn, stimulates proliferation of activated T and B cells expressing IL-2 receptors.

In spite of its potent immunostimulatory effects, the possible use of IL-1 as immunomodulator in humans is hampered by its inflammatory effects. IL-1, indeed, induces a series of typical inflammation- associated metabolic responses, such as fever and prostaglandin G release from cells of hypothalamic thermoregulatory centers, neutrophilia, synthesis of hepatic acute phase proteins, and alteration of glucose homeostasis as well as of the blood levels of divalent cations and corticosterone.

In studies directed at defining possible functional domains within the IL-1 molecule, a synthetic nonapeptide of human IL-1β (VQGEESNDK, position 163-171) was identified which could mimic several of the in vitro and in vivo immunostimulatory activities of the entire protein but was devoid of IL-1 like inflammatory effects.

In our studies the immunorestorative capacities of human recombinant (hu r) IL-1β and of its synthetic fragment 163-171 were compared in BDF1 mice immunodepressed by aging, sublethal irradiation, or both. Subcutaneous administration of hu r IL-1β into immunodepressed mice immediately after carrier priming could restore to normal level helper T cell activity. This was measured by the ability of spleen cells from carrier-primed mice to induce a hapten-specific antibody response in spleen cells from non-immune mice in vitro stimulated with the hapten-carrier conjugate. In parallel, the ability of spleen cells from hu r IL-1β -treated immunodepressed mice to produce IL-2 upon in vitro mitogen stimulation was also increased significantly as compared to that of untreated mice and approached that of immunocompetent controls. The immunorestorative activity of hu r IL-1β on helper T cell activity and IL-2 production could be mimicked by the synthetic nonapeptide 163-171 which, at the doses used, produced in most instances even greater effects than the whole protein. Although the optimal immunorestorative doses of the 163-171 peptide were several orders of magnitude higher than those of hu r IL-1 β , the complete lack of IL-1 like inflammatory effects suggests that the synthetic fragment may be successfully used as immunomodulating agent in the therapy of T cell immunodeficiencies.

Helper T cells recognize foreign antigen in the form of short peptides associated with MHC class II molecules. Since an individual possesses only a limited set of different MHC molecules, each molecule of this set should have the ability to bind a large number of different peptides in order to ensure full immunocompetence. Thus, peptides with unrelated sequences are expected to compete for binding to the same MHC molecule, and this, indeed, has been shown to occur in vitro. We, therefore, examined whether such a competition could also regulate T cell responses in vivo. We found that a synthetic peptide corresponding to residues 46-62 of mouse lysozyme, although not immunogenic itself, effectively inhibits the priming for T cell responses when injected into mice together with foreign protein or peptide antigens. The inhibition observed strictly correlates with the capacity of the competitor to bind to the particular MHC molecule presenting the foreign antigen, and its extent depends on the molar ratio between antigen and competitor.

The preferential recognition of certain amino acid sequences from foreign protein antigens by T cells is referred to as T cell epitope immunodominance. To determine the mechanisms underlying this phenomenon, we have studied the correlation between the interaction of a series of synthetic peptides encompassing the entire hen egg-white lysozyme (HEL) sequence with class II molecules of the H-2^k haplotype, and T cell responsiveness to these peptides. After HEL priming, three immunodominant T cell epitopes were found: two, included in the HEL sequences 51-61 and 112-129, were recognized in association with I-A^k molecules and one, included in sequence 1-18, with I-E^k molecules. Accordingly, these peptides bound to the appropriate class II molecule, as demonstrated by competition for antigen presentation. Several other HEL peptides, although capable of associating with class II molecules, were not immunodominant. The absence of immunodominance has been shown to arise by three different mechanisms: (a) competition by an immunodominant peptide for presentation in vivo, (b) failure to generate the peptide during antigen processing, and (c) an inherently poor capacity of the T cell repertoire to respond to a particular peptide-MHC complex.

IV. Objectives for the next reporting period:

1. Role of interleukins in radiation damage and recovery.

2. Interactions of immunodominant epitopes with MHC molecules in T cell activation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

1. Adorini L., and Sinigaglia F.
I vaccini sintetici.
Biotec 3: 24, 1988

2. Barcellini W., Borghi M.O., Sguotti C., Palmieri R., Frasca D., Meroni P.L., Doria G., and Zanussi C.
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Clin. Immunol. Immunopathol. 47: 147, 1988

3. Frasca D., Adorini L., Landolfo S., and Doria G.
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J. Immunol. 140: 4103, 1988

4. Doria G., Adorini L., Sabbadini E., Mancini C., and Frasca D.
Immunoregulation in aging.
Ann. N.Y. Acad. Sci. (U.S.A.) 521: 182, 1988

5. G. Doria.
Immunoregolazione nell'invecchiamento.
Recenti Progressi in Medicina 79: 310, 1988

6. Adorini L., Sette A., Buus S., Grey H.M., Darsley M., Lehmann P.V., Doria G., Nagy Z.A., and Appella E.
Interaction of an immunodominant epitope with Ia molecules in T-cell activation.
Proc. Natl. Acad. Sci. USA 85: 5181, 1988

7. Adorini L., Muller S., Cardinaux F., Lehmann P.V., Falcioni F., and Nagy Z.A.
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Nature 334: 623, 1988

8. Boraschi D., Nencioni L., Villa L., Censini S., Bossu' P., Ghiara P., Presentini R., Perin F., Frasca D., Doria G., Forni G., Musso T., Giovarelli M., Ghezzi P., Bertini R., Besedovsky H.O., del Rey A., Sipe J.D., Antoni G., Silvestri S., and Tagliabue A.
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J. Immunol. Methods 112: 91, 1988

10. Adorini L., Doria G., and Nagy Z.
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GCI Cortona 1988, Abstract p. 86

11. Romagnoli P., Guttinger M., Adorini L., and Sinigaglia F.
Association between DR haplotypes and antigenic peptides can be predicted by the use of a competitive inhibition assay.
GCI Cortona 1988, Abstract p. 96

12. Doria G., Mancini C., Frasca D., and Adorini L.
Effetto dell'invecchiamento sul meccanismo di attivazione delle cellule T soppressorie.
GCI Cortona 1988, Abstract p. 36

13. Baschieri S., Frasca D., Boraschi D., Bossu' P., Tagliabue A., Adorini L., and Doria G.
Attivita' immunomodulante in vivo dell'interleuchina-1 umana e del suo frammento sintetico 163-171.
GCI Cortona 1988, Abstract p.51

14. Muller S., Adorini L., Appella E., and Nagy Z.A.
Lack of influence of cyclosporine on antigen presentation to lysozyme-specific T cell hybridomas.
Transplantation 46:44S, 1988

15. Sette A., Doria G., and Adorini L.
A basic library of microcomputer programs to obtain immunologically relevant information from protein sequences.
Int. J. Biomed. Computing 22: 165, 1988

16. Adorini L., Appella E., Doria G., and Nagy Z.
Mechanisms influencing the immunodominance of T cell determinants.
J. Exp. Med. 168: 2091, 1988

17. Barcellini W., Meroni P.L., Borghi M.O., Frasca D., Perego R., Doria G., and Zanussi C.
In vivo immunopotentiating activity of thymopentin in aging humans: modulation of IL-2 receptor expression.
Clin. Immunol. Immunopath. 48: 140, 1988

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In vivo restoration of T cell functions by human IL-1 or its 163-171 nonapeptide in immunodepressed mice.
J. Immunol. 141: 2651, 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-060-UK

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. S.B. Field
MRC Cyclotron Unit
Hammersmith Hospital
Ducane Road
GB - London W12 OHS

Telephone number: 01-743 2030 (Ext. 3720)

Title of the research contract:

RBE for normal tissues at low doses and low doses fraction in normal and potentially sensitive populations, with emphasis on parenchymal and vascular damage in late and chronic radiation damage.

List of projects:

1. RBE studies at low doses/fraction on the CNS and the development of vascular related damage.

Title of the project no.: 1

RBE studies at low doses/fraction on the CNS and the development of vascular related damage.

Head(s) of project:

Shirley Hornsey D.Sc., FIBiol, C.Biol.

Scientific staff:

R. Myers PhD, G. Tozer PhD

I. Objectives of the project:

To establish the RBE at low doses/fraction for the CNS. To establish the relationship between parenchymal and vascular damage in the development of late and chronic radiation damage to the CNS following irradiation with X-rays or neutrons. The effect of adjuvant chemicals which may affect the parenchymal or vascular damage selectively or differentially will be used to investigate the pathogenic process and to elucidate factors which may reduce late or chronic radiation damage.

II. Objectives for the reporting period:

1. Completion of drug work assessing the reduction in ataxia by the use of vasoactive drugs.
2. Assessment of damage in drug treated and non-drug treated irradiated spinal cords by leakage of ^{14}C-AIB.
3. Quantitative assessment of the variations in regional blood flow in irradiated cervical cord when computing hardware and software become available.

III. Progress achieved:

1. The cervical spinal cord of rats was treated with 250kV Xrays when the animals were 3-4 months old. After 27Gy Xrays the first ataxic animals were seen at 146 days pos-irradiation (pi) and all animals developed ataxia by 192 days pi. After 25 Gy the first animals developed ataxia by 155 days pi and 83% had developed ataxia by 220 days pi. After 23 Gy the first animal developed ataxia at 179 days pi and 37% developed ataxia by 220 days pi. Other rats were given 27 Gy or 25 Gy Xrays to the cervical cord and were treated with desferrioxamine (30mg in 0.3ml water iv.or sc. 3 times/week, dipyridamole (1.5mg in 0.3 ml in carrier iv. or sc. 3 times/week) or verapamyl (0.75 mg in 0.3 ml carrier iv.or sc. 3 times/week) at 17 weeks pi; those animals treated with desferrioxamine were put on a low iron diet also from 12 weeks pi. The low iron treatment with desferrioxamine had no effect on the haemoglobin levels over the 8 months of administration.

The desferrioxamine with the low iron diet treatment and also treatment with dipyridamole delayed and reduced the onset of ataxia by the equivalent of a dose reduction of 7-8%. Verapamyl had no effect on delaying or reducing ataxia. (1)
Ischemia caused by changes in vascular permeability and blood-flow is associated with the development of radiation necrosis in the CNS. (2) Reperfusion following a transient radiation induced ischemia could exacerbate the radiation damage by reperfusion injury. Superoxide radicals or hydrogen peroxide produced in tissue on reperfusion are reduced to damaging hydroxyl radicals in the presence of iron by the Haber-Weiss and Fenton reactions. Desferrioxamine, a chelating agent, will reduce the free iron in tissue and thus reduce reperfusion injury. The effectiveness of this drug in reducing radiation necrosis is the first evidence that reperfusion injury contributes to radiation damage in the CNS.
Verapamyl is a calcium channel blocking agent and had little if any effect on the development of radiation necrosis. There is some evidence that it is the sodium channels not the calcium channels which are opened in reperfusion injury and the ineffectiveness of this drug suggests this may also be the case for radiation injury. Dipyridamole is a muscle relaxant and its use will increase blood flow. It also reduces the thrombotic activity in blood. It seems likely that both these properties will contribute to the effectiveness of this drug in reducing radiation damage to the spinal cord.

2. An histological study of the irradiated cervical cord in CFHB rats
showed that radiation necrosis was most commonly observed in the white matter of the dorsal columns and the adjacent grey matter of the dorsal horns (2). These two regions of white and grey matter are supplied by a common arterial plexus which has few anastomosis and is noted as being vulnerable to ischemia. The vascular leakage which is the first indication of the pathological changes leading to radiation necrosis occurs predominantly in these two regions. (3) Leakage has been assayed in the various groups by scintillation counting of a segment of spinal cord after innoculation of ^{14}C-labelled α-amino isobuteric acid (AIB). AIB is a small molecule which normally cross the blood brain barrier at a very low rate and is virtually undetectable

in the parenchyma of the normal barried CNS. However, with increased permeability its transfer rate increases when it is rapidly taken up by the parenchyma cells and sequestered thus providing a good marker for leakage in the CNS.
At 8½ months pi 1-2 animals from each group were killed after innoculation of AIB and the spinal cords assayed for leakage by scintillation counting.
No excess leakage was detected in the cervical cords of these assymptomatic animals.

1. Hornsey S, Myers R and Jenkinson T. 1989.
The reduction of radiation damage to the spinal cord by post-irradiation administration of vasoactive drugs.
submitted Int.J.Rad.Onc.Biol.Phys.

2. Myers R, Rogers MA and Hornsey S. 1986
A reappraisal of the roles of glial and vascular elements in the development of white matter necrosis in irradiated rat spinal cord.
Br.J.Cancer 53 suppl.VII 221-223.

3. Hornsey S and Myers R. 1989
Changes in permeability of the blood brain barrier associated with radiation induced white matter necrosis.
submitted Radiotherapy and Oncology

IV. Objectives for the next reporting period:

The analysis and assessment of the variations in regional blood flow by quantitive autoradiography will be completed.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Hornsey Shirley
Experimental Central Nervous System Injury from Fast Neutrons.
In.Radiation Injury to the Nervous System
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pub.Raven press 1989 in press.

Hornsey Shirley, Myers R, Jenkinson T.
The reduction of radiation damage to the spinal cord by post irradiation administration of vasoactive drugs.
submitted Int. J.Radiat.Oncol.Biol.Phys.

Hornsey Shirley, Myers Ralph
Changes in permeability of the blood brain barrier associated with radiation induced white matter necrosis.
submitted Radiotherapy and Oncology

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-061-D

Universität Ulm
Abteilung für Klinische
Physiologie und Arbeitsmedizin
Oberer Eselsberg M 24
D - 7900 Ulm/Donau

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 0731-176 3330

Title of the research contract:

Impairment of the hemo-lymphopoietic cell system and its microenvironment by ionizing radiation. Pathogenesis of non-stochastic and neoplastic effects and conditions for a long term restoration.

List of projects:

1. Mechanisms governing the response of the hemopoietic system and defining its tolerance to partial body and inhomogeneous irradiation.
2. Radiation-induced damage to the stroma - a limiting factor for hemopoietic reconstitution.
3. Pathogenesis of late hemopoietic failure and proliferative disorders in hemopoietic/lymphopoietic cell systems as a consequence of protracted low level radiation exposure.

Title of the project no.: [1]

Mechanisms governing the response of the hemopoietic system and defining its tolerance to partial body and inhomogeneous irradiation

Head(s) of project:

Prof. Dr. W. Nothdurft and Prof. Dr. T.M. Fliedner

Scientific staff:

Dr. K. Baltschukat, Dr. L. Kreja and Prof. Dr. W. Nothdurft

I. Objectives of the project:

It is the objective of this project to investigate in dogs in which way the tolerance of the hemopoietic tissue to radiation exposure is dependent on the fraction of bone marrow irradiated, its localization in the body and the radiation dose absorbed by that volume. The events that have to be investigated in detail are (a) the compensatory response in bone marrow sites that received no damage or less damage than the others, (b) the seeding of circulating stem cells to the damaged bone marrow sites and (c) the recovery processes in the damaged bone marrow.

II. Objectives for the reporting period:

The experimental studies performed in previous years resulted in two important findings which needed in 1988 further investigations expecially with respect to the underlying mechanisms.

(a) The erythroid progenitor cells (BFU-E) from canine bone marrow were found to be extremely radiosensitive in experiments performed with in vitro irradiations.

It is a well known fact that hemopoietic progenitor cell populations are heterogeneous and that colony growth in normal cell populations or after irradiation is highly dependent on hemopoietic growth factors. Thus it was of interest to find out whether colony growth under normal conditions and survival could be increased using different growth conditions.

(b) The survival of BFU-E after in vivo irradiation and their regeneration was studied in dogs which received homogeneous total body irradiation with a dose of 2.4 Gy.

III. Progress achieved:

1. Methodology

(a) Bone marrow samples for the assessment of the BFU-E were obtained from different bones of normal dogs or the irradiated animals by aspiration. For cell separations Ficoll-Hypaque solutions of two different densities, i.e. 1.070 g/ml and 1.077 g/ml were tested simultaneously in some of the experiments. To study the influence of different growth conditions on colony formation of the BFU-E the following recombinant growth factors (= colony stimulating factors) of human origin were added to the cultures: rh-GM-CSF, rh-G-CSF, rh-M-CSF, rh-Il3, rh-Epo and rh-IGF-1. In addition the recombinant GM-CSF and native Il-3 of murine origin were tested, i.e. rm-GM-CSF and m-Il-3. The radition response of BFU-E after in vitro irradiation was assessed with 280 kVp X-rays (HVL = 2 mm Cu; dose rate 0.7 Gy/min). BFU-E were stimulated with rh-Epo (0.5 - 2 U/ml) or BPA containing serum collected from dogs at day 10 after TBI with a dose of 3.9 Gy.

(b) Total body irradiations of dogs were performed with 300 kVp X-rays (HVL = 4 mm Cu; 6.5 cGy/min). A homogeneous dose distribution was obtained by bilateral exposure.

2. Results

(a) Using Ficoll-Hypaque with a density of 1.070 g/ml instead of 1.077 g/ml the cloning efficiency of BFU-E could be significantly improved by a factor of 2 - 4. The colony growth of BFU-E in cultures containing fetal calf serum (FCS) or without FCS was much better when rh-Epo instead of the natural Epo from anemic sheep was used as a specific stimulator. Similar results were obtained for the early multipotent progenitor-cell Mix-CFC. All the other factors tested exhibited no canine BFU-E stimulating activity. The factor rh-IGF -1 was found to potentiate the effect of BPA on BFU-E in suboptimally stimulated cultures.

BFU-E were kept in suspension for 24 hours before irradiation to test whether their radio-sensitivity would be influenced under conditions of enhanced proliferation. First, it was found difficult to extablish appropriate culture conditions that would allow the BFU-E to proliferate in parallel to their differentiation. If the BFU-E were cultured in the presence of FCS and 1-2 U Epo/ml culture medium the best results obtained a recovery of 70% after 24 hours. Second, preliminary results obtained with this method indicate that the radiosensitivity of BFU-E may decrease if the cells are proliferating in suspension cultures under optimal growth conditions.

(b) The most important hematological effects of the acute TBI with a dose of 2.4 Gy were as follows: On the first day after the exposure the lymphocytes had dropped to approximately 20% of the initial average value. The recovery was slow as expected and at day 125 the lymphocyte concentration had just recovered to approximately 50% of the normal value. The granulocyte concentration had reached its nadir at day 8 after TBI, when it had dropped to approximately 25% of the pre-irradiation value. The nadir was followed by an abortive rise and a second decline. The definite recovery of the granulocyte values commenced at day 20 and at day 40 the values were back in the normal pre-irradiation range. The granulocyte/macrophage progenitor cells (GM-CFC) in the bone marrow had decreased to between 3% and 7% of the initial concentration in different sites, i.e. the humeri, the sternal bones and the iiac crests. In all bone marrow sites the recovery was fast in the period between day 14 and day 28. However, thereafter the GM-CFC values remained at a subnormal level, i.e. between 50% and 86% of the pre-irradiation values up to day 125. The BFU-E concentration in the different bones was extremely reduced at day 1 after exposure to between 0.2% and 3% of the initial values. These values are compatible with their high radiosensitivity, as compared to the GM-CFC. The values remained rather low up to day 14. Thereafter in the period up to day 28 the BFU-E concentration showed a considerable increase. However, up to day 125 the BFU-E values in all the bone remained at a clearly subnormal level as found for the GM-CFC.

3. Discussion

Significant progress was achieved in the field of canine progenitor cell characterization, especially of BFU-E but also Mix-CFC. Just the improvement in their cloning efficiencies in primary cultures allows more detailed analyses in sequential studies in dogs after different conditions of radiation exposure as well as radiobiological studies in vitro. However, the conditions to support and maintain the growth of BFU-E in suspension culture obviously are still suboptimal. The results obtained in the sequential studies in dogs which had been exposed to homogeneous TBI with a radiation dose of 2.4 Gy clearly show that the regeneration of the BFU-E in the bone marrow is significantly delayed. There is some residual damage which is in accordance with similar observations in the compartment of the GM-CFC from previous studies.

IV. Objectives for the next reporting period:

The further research will be directed to the following topics: It will be important to improve the conditions in the suspension cultures that will allow the maintenance of BFU-E and Mix-CFC and their proliferation for several days for further radiobiological studies. Different recombinant growth factors of human origin such as r-erythropoietin or r-IL-3 will be used as stimulants alone or in combination with canine serum or accessory cells (lymphocytes and monocytes). The determination of the radioresponse characteristics of the canine pluripotent progenitor cell Mix-CFC will be of special interest for purposes of intercomparisons of progenitor cell radiosensitivity between species. In vivo studies will directed to sequential analyses of BFU-E changes in dogs after homogeneous total body irradiation with moderate doses.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Our Ulm research group is an active member of the European Late Effects Project Group, in which 19 Europeean research laboratories "compare notes" on these irradiation studies.

VI. Publications:

1. Publications in scientific journals

Baltschukat, K., W. Nothdurft and T.M. Fliedner (1988)
Hematological effects in dogs after irradiation of the lower part of the body with a single myeloablative dose.
Radiother. Oncol. 13, in press

Fliedner, T.M., W. Nothdurft and K.H. Steinbach (1988)
Blood cell changes after radiation exposure as an indicator for hemopoietic stem cell function.
Bone Marrow Transplantation 3, 77-84

Fliedner, T.M., and K.H. Steinbach (1988)
Repopulating potential of hematopoietic precursor cells.
Blood Cells 14, 393-410

Fliedner, T.M., K.H. Steinbach, T. Szepesi (1988)
Hematological indicators in the determination of clinical management strategies in radiation accidents.
In: Zhang Q-X, Wu D-C (eds.) Radiation biological effects. Modifiers and treatment. Proceedings of the International Conference on Biological Effects of Large Dose Ionizing and Non-Ionizing Radiation, Hangzhou, March 29 - April 1, 1988, Society of Radiation Medicine and Protection, Chinese Medical Association, Beijing, pp 60-91

Nothdurft, W., K. Baltschukat und T.M. Fliedner (1988)
Untersuchungen über die Kompensationsmechanismen und Regeneration der Hämopoese nach einzeitiger Halbkörperbestrahlung im Tierexperiment an Hunden.
Radiobiol. Radiother. 29, 334-336

Szepesi, T., T.M. Fliedner and K.H. Steinbach (1988)
Hematological responses after accidental exposure to ionizing radiation: a review of 22 reported accidents.
In: Zhang Q-X, Wu D-C (eds.) Radiation biological effects, Modifiers and treatment. Proceedings of the International Conference on Biological Effects of Large Dose Ionizing and Non-Ionizing Radiation, Hangzhou, March 29 - April 1, 1988, Society of Radiation Medicine and Protection, Chinese Medical Association, Beijing, pp 30-59

2. Short communications, abstracts

Fliedner, T.M. (1988)
Hematological indicators to predict patient recovery after whole-body irradiation as a basis for clinical management.
International Conference: The medical basis for radiation accident preparedness: II. Clinical experience and follow-up since 1979, Oak Ridge, 19-23 October, 1988

Title of the project no.: 2

Radiation induced damage to the stroma - a limiting factor for hemopoietic reconstitution?

Head(s) of project:

Prof. Dr. W. Nothdurft

Scientific staff:

Prof. Dr. T.M. Fliedner, Dr. L. Kreja, Prof. Dr. W. Nothdurft

I. Objectives of the project:

The various cell types forming the cellular stroma and its extracellular matrix in the hemopoietic organs are considered rather resistant to radiation with respect to acute desintegration. However, late damage to the stromal cells is of importance resulting in functional and structural alterations that again may lead to bone marrow hypoplasia or even aplasia. Therefore, the sensitivity of the stroma to ionizing radiation and its impact on the hemopoietic supportive function will be studied using modern in vivo and in vitro assay systems.

II. Objectives for the reporting period:

The research was directed to two aspects to characterize the biological properties of the stroma and its impact on hemopoietic funnction. First, it was of interest to further study the stromal progenitor cells CFU-F in vitro with respect to the requirement for certain growth factors and to define their capacity for sublethal damage repair after in vitro irradiation. Second, the research directed to the establishment and standardization of long-term cultures with canine bone marrow cells according to the Dexter system was continued.

III. Progress achieved:

1. Methodology

To obtain cell suspensions appropriate for CFU-F cultures bone marrow cell suspensions were separated using the buffy coat method. The nucleated bone marrow cells were inoculated in liquid cultures at different concentrations to establish dose response curves for colony growth. Colony formation was studied under the influence of the growth factors of interest.

The capacity of CFU-F for repair of sublethal damage after in vitro irradiation was tested in the following way using the split-dose technique: The cells were kept in suspensions at appropriate concentrations and irradiated at room temperature with increasing doses in the range from 0.36 Gy to 9.0 Gy. Cell suspensions that had received a dose of 2.9 Gy were kept at either room temperature or 37° C for 2 hours to allow possible recovery from the first dose and then were again exposed to incremental doses to establish a survival curve.

Long-term cultures of canine bone marrow cells were prepared in the following way. The cells were collected by aspirations or harvested from biopsy materials. In addition cultures were prepared with cryopreserved canine fetal liver and spleen cells. The influence of several growth factors on the maintenance of hemopoietic activity was tested by means of progenitor cell determinations.

2. Results

(a) Canine CFU-F colony formation in the presence of human or porcine-platelet-derived growth factors (PDGFs) was assayed over a culture period of more than 14 days For both factors no significant improvement of CFU-F growth could be found when compared to the standard culture conditions.

The survival curves of the CFU-F after single doses were characterized by D_0 values of approximately 2.2 Gy and an extrapolation number $n \sim 1.3$. Thus, the curves exhibited a small shoulder equivalent to a value of $D_q \sim 0.5$ Gy.

The survival data obtained for the CFU-F after the second irradiations were characterized by the following features: Generally the CFU-F number had decreased 2 hours after the first dose to approximately 60% of the original survival fraction independent of the incubation temperature in the interval between the two fractions. However, the slopes of the survival

curves obtained after the second irradiations were rather different. Those cells kept at 37° C showed a much shallower slope than the cells that were irradiated only once. On the other hand, the cells that were kept at 20° C between the two irradiations showed a slope rather similar to that obtained after the single exposure.

Thus, there is in principle some recovery from sublethal damage repair of the CFU-F. However, it is masked by interfering effects possibly due to suboptimal culture conditions.

(b) In suspension cultures with canine bone marrow cells in which an adherent cell layer was established the maintenance of granulocyte/macrophage progenitor cells (GM-CFC) could be shown up to 6 to 8 weeks after the begin of the incubation. The best results were obtained with cultures that were supplemented with 10% dog serum and 10% horse serum and additionally contained hydrocortisone (10^{-5}). No significant improvement of hemopoietic activities could be achieved under the influence of several factors which under certain conditions may exhibit stimulatory activities on hemopoietic cells such as PDGF, serum from lethally irradiated dogs, β-xyloside, α-thioglycerol or mercaptoethanol. In cultures which were prepared from cryopreserved fetal liver cells or fetal spleen cells GM-CFC were found over a period of 3 to 4 weeks but thereafter they disappeared.

Up to now it was not possible to establish for canine bone marrow cells a long-term culture system that allowed the maintenance of hemopoietic activity for longer than 10 weeks, as is the case with murine or human bone marrow in the Dexter culture system.

3. Discussion

The results obtained for canine CFU-F after split-dose irradiation indicate some capacity of sublethal damage accumulation and repair. However, there is some loss of CFU-F after the first dose for unknown reasons. Since in the suspension cultures of canine bone marrow cells hemopoietic activity could be maintained over some 6 to 8 weeks at best the culture conditions according to the Dexter technique as applied for human and murine bone marrow obviously are not appropriate for canine cells. It is an open question whether this is due to the lack of certain cells in the adherent layer essential for hemopoietic cell growth or whether some factors are missing in the culture medium.

IV. Objectives for the next reporting period:

The further research will be directed to the following aims: In the area of methodological studies several modifications will be tested that might help to improve the growth conditions of hemopietic cells in long-term suspension cultures. Such experiments will include the use of canine sera of different qualities and endothelial cell adherent layers. The CFU-F will further be characterized with respect to their radiobiological properties concerning recovery from sublethal damage and potentially lethal damage repair. Furthermore, sequential CFU-F determinations will be performed in vivo in dogs after total body irradiation with moderate radiation doses to correlate the progenitor cell changes in the stroma with those in the hemopoietic system.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

There is an active collaboration within the European Late Effects Project Group and its 19 research institutions.

VI. Publications:

1. Publications in scientific journals

Kreja, L., K. Baltschukat and W. Nothdurft (1988)
Growth of erythroid burst forming units BFU-E in cultures of canine bone marrow and peripheral blood cells. Effect of serum from irradiated dogs.
Exp. Hematol. 16, 647-651

Nothdurft, W., K. Baltschukat and T.M. Fliedner (1988)
Hematological effects in dogs after sequential irradiation of the upper and lower part of the body with single myeloablative doses.
Radiother. Oncol. 13, in press

2. Short communications, abstracts

Fliedner, T.M., W. Weinsheimer, W. Nothdurft and W. Calvo (1988)
Bone marrow structure in its significance for the regulation of hematopoiesis.
XXII Congress of the International Society of Hematology, Milano, August 22 - September 2, 1988. Book of abstracts, p. 155

Kreja, L., K. Baltschukat and W. Nothdurft (1988)
In vitro studies on the X-ray radiosensitivity of canine bone marrow erythroid burst forming units (BFU-E) and fibroblast forming units (CFU-F).
3. Symposium Molekulare und zelluläre Mechanismen der biologischen Strahlenwirkung, München-Neuherberg, 23.-25. März 1988. Book ob abstracts

Title of the project no.: 3

Pathogenesis of late hemopoietic failure and proliferative disorders in lymphopoietic cell systems as a consequence of protracted low level radiation exposure.

Head(s) of project:

Prof. Dr. T.M. Fliedner and Prof. Dr. H.J. Seidel

Scientific staff:

Prof. Dr. W. Calvo, Dr. L. Kreja, Dr. A. Ingendaay, Dr. D. Zinser

I. Objectives of the project:

It is the objective of this project to analyse the pathogenetic mechanisms that lead to the development of leukemia and/or aplastic anemia in response to chronic low level ionizing radiation. As experimental models the dogs studied at Argonne under chronic total body irradiation were considered and small laboratory animals at Ulm.

II. Objectives for the reporting period:

a) In 1988 an attempt was made to analyse granulocyte recovery in accidental protracted radiation exposure in humans with respect to pathogenetic mechanisms at the stem cell level.

b) Development of a mice model to compare low level protracted radiation exposure to low level protracted chemical exposure.

III. Progress achieved:

a) As far as the human radiation accident data is concerned, we obtained the detailed hematological data from the Algerian accident. In this accident situation 4 persons were exposed to total body radiation doses beween 862 and 1015 Gy during 38 days. Thus the daily dose amounted to 22.9 - 27.5 Gy. The radiation exposure originated from a 192 radium source. The granulocyte values were ploted and it was found that at the end of this exposure a severe granulocytopenia was observed. These patients were treated and a spontaneous hemopoietic recovery commenced around 35 days after the end of exposure and 75 days after the beginning of the exposure.

On the basis of a biomathematical model of granulocyte regulation it was possible to simulate the granulocyte recovery curves after this type of protracted irradiation. It was necessary to modify a previous biomathematical model of granulocytopoiesis by deviding the stem cell pool into two subpopulations, one with a unlimited replicated potential and a second one with a limited replicative potential. Under these circumstances it was found that the damage to the stem cell pool amounted to a remaining intact stem cell population of 0.006%. This is equivalent to an acute radiation exposing the organism to a total dose of about 400 Gy. Under these circumstances the granulocyte recovery pattern is compatible using the biomathematical model with the remaining intact stem cell pool of 0.008%.

These findings indicate that even after protracted irradiation it is decisive whether a hemopoietic stem cell population remains from which a spontaneous hemopoietic recovery can commence.

Further studies are indicated to determine the limits of hemopoietic regeneration using pathophysiological information on hemopoietic stem cell recovery potentials.

b) In previous studies invetigating protracted radiation exposure of dogs it was found that there is initially after such a prolonged radiation period a decrease in the hemopoietic stem cell pool.

In order to initiate an investigation on the pathogenetic mechanisms of low level protracted radiation exposure to low level protracted chemical exposure (organic solvents such as benzene) a model of benzene exposure of mice was established. This comparative study is important to analyse the specificities of radiation exposure as compared to chemical exposure. After 16 weeks of exposure to 300 ppm of benzene and almost 2 years of observation almost all mice exposed to benzene have developed malignancies, mainly hemolymphopoietic tissue. Doses of 30 to 900 ppm have been applied to study dose-response effects at the level of hemopoietic stem cells.

This has been established now and further protocols with combination of benzene and other toxic substances such as radiation can be planned.

IV. Objectives for the next reporting period:

a) It is envisaged that further cases of the effects of protracted radiation exposure to the hemopoietic tissue will be investigated with respect to the pathophysiological mechanisms of tolerance to low level protracted radiation exposure. The endpoints will be hemopoietic blood cells and the relevant hemopoietic stem cell populations. Such studies will be important to develop early prognostic indicators for late consequences.

b) The studies in mice using chemicals and radiation to study the development of neoplastic or non-neoplastic late effects after protracted low level exposure will be continued. It is important to develop early indicators to predict late neoplastic developments. Such studies will require the utilization of molecular biology technologies such as the Southern and the Northern Blot techniques.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

It is greatfully acknowledged that French, Chinese and Russian, as well as South American groups have signaled their support and collaboration in these studies. Furthermore, the scientific communications with investigators in the United States of America, such as the Brookhaven National Laboratory and the Argonne National Laboratory will continue. Finally, in Europe the membership of our group in the European Late Effects Project Group is very instrumental to establish molecular biology techniques in radiation reseach.

VI. Publications:

Fliedner, T.M., K.H. Steinbach, T. Szepesi (1988)
Hematological indicators in the determination of clinical management strategies in radiation accidents.
In: Zhang Q-X, Wu D-C (eds.) Radiation biological effects. Modifiers and treatment. Proceedings of the International Conference on Biological Effects of Large Dose Ionizing and Non-Ionizing Radiation, Hangzhou, March 29 - April 1, 1988, Society of Radiation Medicine and Protection, Chinese Medical Association, Beijing, pp 60-91

Szepesi, T., T.M. Fliedner and K.H. Steinbach (1988)
Hematological responses after accidental exposure to ionizing radiation: a review of 22 reported accidents.
In: Zhang Q-X, Wu D-C (eds.) Radiation biological effects. Modifiers and treatment. Proceedings of the International Conference on Biological Effects of Large Dose Ionizing and Non-Ionizing Radiation, Hangzhou, March 29 - April 1, 1988, Society of Radiation Medicine and Protection, Chinese Medical Association, Beijing, pp 30-59

H.J. Seidel, L. Weber, D. Zinser: Benzene-Toxicity-Experimental Studies with Mice and Safety-Regulations in the Federal Republic of Germany. Int. Conf. on Safety and Health, Beijing, VR China, Oct. 1988, p. 609-615

A. Ingendaay, H.J. Seidel: The effect of bennzene and its metabolites on fibroblast colony formation. ISH Conf. Milan, 1988 (Abstract)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-082-UK

Berkeley Nuclear Laboratories
Central Elect. Generating Board
Berkeley
GB - Gloucestershire GL13 9PB

Head(s) of research team(s) [name(s) and address(es)]:

Dr. T. Healey
Berkeley Nuclear Laboratories
Central Elect. Generating Board
Berkeley
GB - Gloucestershire GL13 9PB

Telephone number: 0453-810451

Title of the research contract:

The establishment of radiological protection criteria for non-uniform skin exposure.

List of projects:

1. A cooperative study to establish radiation protection criteria based on experiments on pig and mouse skin.

Title of the project no.: BI6-C-082-UK

A cooperative study to establish radiation protection criteria for non-uniform skin exposure based on experiments on pig and mouse skin.

Head(s) of project:

M W Charles

Scientific staff:

M W Charles
J Wells

I. Objectives of the project:

A collaborative programme will investigate non-stochastic effects in mouse and pig skin and stochastic effects in mouse skin, following uniform and non-uniform alpha and beta irradiation. This should enable radiological protection criteria to be developed for limiting non-uniform skin exposure and will provide the design parameters for personal skin dosemeters. In particular the aim is to identify the nature and depth of cells at risk in the skin and to identify an appropriate area over which dose should be averaged.

II. Objectives for the reporting period:

Production of large area (8 cm^2) Cm-244 alpha and Tm-170 beta sources to carry out large area uniform and non-uniform irradiations of pig and mouse skin using the automated extrapolation chamber facility for detailed isodose determinations on beta radiation sources. Comparison of extrapolation chamber and thermoluminescence dosimetry of beta sources. Collation of stochastic and non-stochastic animal data. Initiate studies to obtain high LET RBEs for non-stochastic effects in pig and mouse skin and skin cancer (and cataract) induction in the mouse.

III. Progress achieved:

1. Methodology

The study of high LET RBEs for non stochastic effects and skin cancer induction in mouse skin (and cataract) has been extended through the use of neutron irradiations as well as the alpha irradiations commenced last year.

Two large (8cm^2) Cm-244 alpha sources (total activity 0.1 mCi) have been mounted in irradiation jigs for mouse skin exposures. The suitability for use of these sources on pig skin has been confirmed through the use of mock sources on shaved pig skin. No damage from hair bristles was observed in the protective plastic layer.

The 24 keV filtered neutron facility at the Harwell PLUTO reactor has been used for 100 mouse skin exposures which at this energy are effectively delivered to half the body. Doses of about 0.3 Gy were used.

Discussions have taken place through EURADOS committee 2 with Dr Herbaut (CEA, Grenoble, France) and Dr Patau (Univ of Tolouse, France) to ascertain the feasibility of a collaborative dosimetry project. This will compare measured and calculated (Monte Carlo) beta doses from Co-60 neutron activated particles, of great practical relevance to the nuclear power industry.

Work on some non-stochastic aspects of this contract continue to be coordinated through a EULEP Skin Task Group.

The computer controlled extrapolation chamber (based on a BBC micro-computer) has been extended to enable automatic generation of full isodose date. The performance of this facility remains to be fully evaluated.

Non-linear regression analysis of skin cancer dose response data has been transferred from a BBC to an IBM compatible micro-computer.

As a result of membership of the ICRP Skin Radiobiology Task Group the results of this project have been discussed and are currently influencing the derivation of radiological protection criteria for skin exposures by the ICRP and the NCRP.

2. Results

Radioactive source production has continued successfully with personal extremity doses being maintained at low levels (< 5 mGy). Some minor contamination problems have been encountered with the high activity Cm-244 alpha sources. This has been remedied by re-design of the irradiation jigs to provide greater tolerance clearances to avoid abrasion of the sources. The sources have been further protected by a 4 micron mylar plastic film.

Analysis of mouse skin cancer data has been extended down to the lowest doses of 2 Gy and continues to indicate a linear, no threshold response for doses below about 20 Gy.

Non-stochastic effects data for the pig indicates 2 temporal phases of dermal atrophy following Sr/Y-90 and Tm-170 beta exposures. This confirms the need to evaluate the skin dose at 2 depths in order to provide a prognosis for an acute skin exposure.

In the case of skin exposure from small radioactive particulates (hot particles) the maintained use of an averaging procedure (over 1 cm^2) for radiological protection has been proposed, in contrast with a recent NCRP proposal to limit exposure on the basis of a limit on the total number of beta particles emitted from a particle.

3. Discussion

Recent human data has emphasised the potential importance of radiation induced skin cancer and it may be possible that this will prove to be the limiting end point for large area exposures involving the majority of human body skin. While still the subject of contoversy the basal cells of the skin are likely to remain identified as the cells at risk in this case. For small area exposures the non-stochastic end points will be of most relevance and the cells at risk will lie in the dermis. For hot particle exposures a dose limit of 0.5 Gy over 1 cm^2 at a level in the dermis should prevent detrimental effects. Details of a final protection protocol for the skin to cover all practical exposure situations is the subject of current active discussions within ICRP and NCRP but should be resolved within the next 2 years. The application of such criteria to specific important practical problems such as Co-60 hot particles (known as 'fleas' in the US) requires further development in dosimetry of real particles. It is a problem of sufficient importance to require separate animal studies. These will be commenced in the final phase of the current project.

IV. Objectives for the next reporting period:

Further high activity Sr/Y-90 and Tm-170 beta sources will be produced to facilitate a study of the dependence of sensitivity to skin cancer induction on mouse strain and for wound healing studies in pig skin.
Non-uniform alpha exposures of mouse skin will be carried out using plastic sieve absorbers on large area Cm-244 sources.
Further intermediate energy neutron exposures of mouse skin will be carried out on the basis of interim data from the pilot study.
The performance of the automated extrapolation chamber in its full isodose mode will be evaluated.
Data on stochastic and non-stochastic effects in pig and mouse skin will be utilised to provide guidance to the ICRP and NCRP to aid the development of radiological protection criteria for skin.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr J W Hopewell, Churchill Hospital, Oxford
Dr J E Coggle, St Bart's Medical College, London
Dr Y Herbaut, CEA, Grenoble
Dr P Patau, University of Tolouse

VI. Publications:

Skin Carcinogenesis Following Uniform and Non-Uniform Beta Irradiation, 1988. M. W. Charles, J. P. Williams and J. E. Coggle
25th Hanford Life Science Symposium. Health Physics 55(2) 399-406

Skin Carcinogenesis Following Uniform and Non-Uniform Beta Irradiation, 1989. M. W. Charles, J. P. Williams and J. E. Coggle
CEGB Report, Berkeley Nuclear Laboratories, RD/B/R6122/R89

Extrapolation Ionisation Chamber Measurements on Beta Emitting Sources Provided for the CEGB Collaborative radiobiology Programme, 1988.
J. Wells
CEGB Report, Berkeley Nuclear Laboratories, RD/B/R0867/R88

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Paterson Laboratories
Christie Hospital
and Holt Radium Institute
Wilmslow Road
GB - Manchester M20 9BX

Contract no.: BI6-C-062-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.H. Hendry
Paterson Laboratories
Christie Hosp. & Holt Radium Inst.
Wilmslow Road
GB - Manchester M20 9BX

Telephone number: 061-445 8123

Title of the research contract:

Cellular analysis and dose-response relationships in long-term radiation injury to mouse bone marrow.

List of projects:

1. Determination of long-term injury of radiation to mouse bone marrow.

Title of the project no.:

Determination of longterm injury of radiation to mouse bone marrow

Head(s) of project:

J H Hendry

Scientific staff:

J H Hendry, N G Testa, J Bierkens, M C Baird, D Y Qi

I. Objectives of the project:

To investigate residual haemopoietic injury in terms of deficiencies in various progenitor and maturing cell populations in mice, after irradiation delivered acutely at a low dose rate or using various fractionation regimes.

II. Objectives for the reporting period:

(A) To measure the radiosensitivity of different precursor cells in the haemopoietic hierarchy in the dog and in man using specific growth factors.

(B) To study the acute and longterm effects of various doses and regimens of irradiation on the haemopoietic stroma, using longterm bone marrow cultures.

III. Progress achieved:

(A) i. Cultures of γ-irradiated canine bone marrow cells were grown for 10 days using different colony-stimulating factors (CSF) at optimal concentrations. These included 10% serum (DS) from dogs irradiated 9 days previously with 3.9 Gy (kindly provided by Dr W. Nothdurft, Ulm University) together with 10^6 white blood cells (WBC) per ml, 15% PHA-conditioned medium (CM), 20% 5637-CM, 3000 U/ml human rGM-CSF, and 5000 U/ml human rG-CSF.

Sensitivity parameters are shown in Table 1. There was no significant shoulder on the survival curve using DS + WBC or 5637-CM, but there was with PHA-CM, measured as the mean of values in several experiments. The curves determined using rGM-CSF and rG-CSF showed significant shoulders but the latter was assessed in only one experiment. Average values of colony-forming efficiency (CFE) were similar when using different factors, and Do values were about 50 cGy (up to 70 cGy) or less. There was no clear relationship between CFE and Do over an extreme range of about 7 in values of CFE. Cluster (2 to 49 cells) : colony ($\geq$ 50 cells) ratios increased from about 1 in controls to about 5 after 1 Gy. These studies confirm other data in the literature using irradiated dog serum as a source of stimulator which show that progenitor cells in the dog are more radiosensitive than in mouse or in man (see below).

Table 1. Radiosensitivity of haemopoietic progenitor cells

	CSF	CFE %	Do ± sem (cGy)	n
Dog	DS + WBC[b]	0.05	50 ± 5	1.9 ± 0.4[a]
			(71 ± 7	1.0)
	PHA-CM	0.08	41 ± 4	2.0 ± 0.3
			(64 ± 3	1.0)
	5637-CM	0.06	40 ± 5	1.5 ± 0.3[a]
			(50 ± 2	1.0)
	rhGM	0.05	33 ± 6	2.2 ± 0.7
			(52 ± 4	1.0)
	rhG[b]	0.06	21 ± 5	6.2 ± 3.8
			(52 ± 11	1.0)
Man	h5637-CM	0.09	138 ± 12	1.0 ± 0.1[a]
			(130 ± 4	1.0)
	rhIL-3	0.14	124 ± 16	1.0 ± 0.1[a]
			(122 ± 6	1.0)
	rhGM[b]	0.14	115 ± 15	1.4 ± 0.2[a]
			(138 ± 7	1.0)
	rhG[b]	0.08	100 ± 21	1.0 ± 0.3[a]
			(100 ± 8	1.0)

[a] = not significantly different from 1.0
[b] = 1 experiment only

ii. Human haemopoetic cells are obtained from rib samples or aspirates. Optimal concentrations of the human CSF were lower for human CFC than for dog CFC, being 1000 U/ml rhGM-CSF or G-CSF, 500 ng/ml rhIL-3, and 10% h5637-CM. The colonies were counted between days 9 and 12 of growth. All the survival curves were exponential, and there was no significant correlations between radiosensitivity and colony-forming efficiency (CFE) among different experiments. Mean values of the survival parameters among several experiments are quoted in Table 1, although there was a significant difference between experiments in the case of IL-3 and G-CSF. The use of rhG-CSF gave the lowest Do value of 100 cGy.

A comparison of these data for dog and man with those for mouse (1987 progress report) shows the following features:

(a) The human CSF had a higher activity on human than on dog CFC,

(b) A higher degree of biological variation was evident in human samples than in mouse or dog samples,

(c) Different proportions of CFC are stimulated by the same CSF in different species, and some take longer to produce colonies,

(d) The CFC stimulated in dog bone marrow are more radiosensitive compared to human and mouse, and the latter show some similarities in radiosensitivity,

(e) G-CSF recruits the most radiosensitive subpopulation in all 3 species, and in general radiosensitivity increases with differentiation status.

(f) Among the different experiments with a given CSF, the radiosensitivity was not related to the CFE,

(g) The increase in cluster:colony ratio with increasing dose was more marked in the case of dog than the mouse, and this may reflect or be the cause of the higher apparent sensitivity of haemopoeitic cells in the dog.

(B) Assessment of residual stromal damage after whole body irradiation and its repair in time was performed using long-term bone marrow cultures (LTBMC). Bone marrow from 'in vivo' irradiated BDF_1 mice was explanted in T25-culture flasks (Dexter - conditions) at various times post-irradiation: 1 day, 1, 3, 7 and 12 months. The different doses were chosen so as to cover a wide range of <u>therapeutic</u> irradiation

regimens: 4.5 Gy and 10 Gy at low dose rate (1.6 cGy/min); 10 Gy at low dose rate (0.05 cGy/min); a fractionated regimen of 4 x 4.5 Gy with 3-weeks intervals.

Preliminary results show a correlation between 'in vivo' irradiation doses and the proliferative capacity of the stroma to form an adherent layer supportive of haematopoiesis. After 3 months a near recovery of the stroma was seen in LTBMC established from 4.5 Gy irradiated mice, as measured by both the adherent stromal cell number and the non-adherent cumulative haemopoietic cell production. However, the recovery of early haematopoietic precursor cells in these cultures lagged behind the apparent recovery of the stroma to support haematopoietic cell output: $\sim$50% recovery of CFU-S and $\sim$75% recovery of GM-CFC. At 7 months the recovery of CFU-S and GM-CFC number in LTBMC from 4.6 Gy and low-dose-rate 10 Gy-irradiated mice seems to reach completion. High-dose-rate 10 Gy and 4 x 4.5 Gy cultures remain suboptimal after 7 months. In the 4 x 4.5 Gy cultures no fully-confluent adherent layer and only a few % of normal CFU-S were found after 7 months.

IV. Objectives for the next reporting period:

1. To further study longterm effects of various doses and regimens of irradiation on the haemopoietic stroma, using longterm bone marrow cultures.

2. To study the interaction of irradiation and chemotherapeutic agents for the production of longterm haemopoietic injury.

3. To investigate the radiosensitivity of bone marrow stem cells in foetal and neonatal mice.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

C. Tejero, Dept of Bioquimica, Universidad Complutense de Madrid, Facultad de Veterinaria, Cuidad University, 28040 Madrid, Spain, and other EULEP groups for discussions.

VI. Publications:

GALLINI, R., HENDRY, J.H., MOLINEUX, G. & TESTA, N.G. (1988). The effect of low-dose rate on recovery of hemopoietic and stromal progenitor cells in mouse bone marrow. Radiation Research, 115: 481-487.

TESTA, N.G., HENDRY, J.H. & MOLINEUX, G. (1988). Long-term bone marrow damage after cytotoxic treatment: stem cells and microenvironment. In: Hematopoiesis: Long-term effects of chemotherapy and radiation.(Eds.N.G. Testa, R.P.Gale. Marcel Dekker, New York and Basel. Hematology 8: 75-91.

TEJERO, C., LORD, B.I., MASON, T.M. & HENDRY, J.H. (1988). Long-term haemopoietic injury in mice after repeated irradiation: precursor-cell cycling and its regulation. European Journal of Haematology, 278-284.

TEJERO, C., HENDRY, J.H. & TESTA, N.G. (1988). Persistent dose-dependent increases in cycling of haemopoietic precursor cells after irradiation. Cell and Tissue Kinetics, 21: 33-43.

TEJERO, C., TESTA, N.G. & HENDRY, J.H. (1988). Decline in cycling of granulocyte-marcophage colony-forming cells with increasing age in mice. Experimental Haematology. In press.

BAIRD, M.C., HENDRY, J.H. & TESTA, N.G. Radiosensitivity differences among haemopoietic progenitor cells selected using recombinant growth factors. In preparation.

BAIRD, M.C. (1988). The radiosensitivity of haemopoietic cells in different species. PhD thesis, University of Manchester.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-063-UK

Churchill Hospital
Research Institute
University of Oxford
Headington
GB - Oxford OX3 7LJ

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Hopewell
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Telephone number: 0865-74.18.41

Title of the research contract:

Early and late effects of radiation on the skin.

List of projects:

1. Pathogenesis of early and late radiation damage to skin.
2. The biological effects of non-uniform irradiation on pig skin.

Title of the project no.: 1

Pathogenesis of early and late radiation damage to skin

Head(s) of project:

Dr. J.W. Hopewell

Scientific staff:

Dr. M. Rezvani
Dr. G. van den Aardweg
Dr. P. Mortimer
Mr. M. Baker

I. Objectives of the project:

The pathogenesis of early and late radiation damage to the skin is being studied in the pig. The results obtained from experiments in this species are likely to provide data which may be more readily extrapolated to man.

II. Objectives for the reporting period:

In the period between irradiation and the development of dermal atrophy, or even ischaemic dermis necrosis after high doses or X-rays, there is a phase of clinically identifiable dermal oedema. Time-related changes in lymphatic clearance have been assessed using a ^{99m}Tc-rhenium sulphate colloid clearance technique.

III. Progress achieved:

In the past, the clearance of radio-labelled proteins has been used to assess dermal lymph-flow in patients. However, the applicability of the results has been limited by the apparent variability in the measurements. This has been attributed to the type of tracer used and to variations in the local injection technique. Prior to studies being carried out in irradiated skin investigations have been carried out in normal pig skin in order to standardise the technique and to minimise the variability in the measurements of lymph-flow.

The flank skin of 12 week old Large White pigs was used in these investigations and the effectiveness of two tracers, ^{131}I-human serum albumen (^{131}I-HSA) and ^{99m}Tc-rhenium sulphide colloid, was tested. The tracers were injected into the superficial dermis and their disappearance from the site of injection was monitored by a NaI detector. The resulting data which indicated a very slow rate of clearance of both tracers was best fitted by an equation which included a single exponential plus a constant term. The results obtained with ^{99m}Tc-colloid were comparable with those for ^{131}I-HSA, perhaps not surprising since the particle size of the two tracers was similar, 5 - 7nm and 7.5nm, respectively. There was no influence of the volume of tracer injejcted (0.03ml - 0.3ml), however, the rate of clearance was dependent on the data collection time, faster half-clearance times for the tracer were associated with short periods of data collection. For the subsequent studies in irradiated and normal skin the ^{99m}Tc-colloid was used, 0.03ml of the tracer was injected and the data collection time was standardised at 30min. The half-clearance time of the tracer in normal skin using these standard conditions was ~0.8hr.

Using the standard conditions listed above, the effects of the depth of injection on the clearance rate of the ^{99m}Tc-colloid was investigated. The previous sub-epidermal injection site was compared with a deep dermal and a subcutaneous injection. This demonstrated that the sub-epidermal injection was cleared significantly faster (mean half-clearance time 0.79 ± 0.3hr; n = 19) than the subcutaneous injection (half-time 2.64 ± 0.34hr; $p<0.001$) and the deep dermal injection (half-time 2.74 ± 0.5hr; $p<0.001$). There was also a greater consistency in the measurements obtained after the sub-epidermal injection. This site of administration of the tracer was used for the study of changes in lymph-flow after irradiation.

Prior to irradiation six fields, measuring 4cm x 4cm and separated by a 6cm gap, were marked out on the left flank of each pig. Each field was irradiated with a single dose of 18Gy of 250kV X-rays. At intervals of 3, 6, 9, 12, 26 and 39 weeks after irradiation lymph flow was assessed in the centre of each irradiated site. Simultaneous measurements were made in areas of unirradiated skin midway between the irradiated fields.

The time related changes in lymph clearance in irradiated and the adjacent areas of unirradiated skin are shown in Figure 1. A signficant transient impairment of lymph clearance was found at 6 - 12 weeks after irradiation; the significant impairment at 6 weeks was prior to the clinical appearance of oedema at ~9 weeks. Impairment in lymph clearance was also seen in unirradiated skin at 9 and 12 weeks. This possibly reflects the pattern of lymphatic flow through the dermis, across the flank, to the regional lymph nodes.

These results suggest that a phase of increased vascular permeability precedes the reduction in blood flow that has previously been reported at 9 - 12 weeks. The blood resulting from this permeability change may be an additional factor contributing to the subsequent reduction in blood flow.

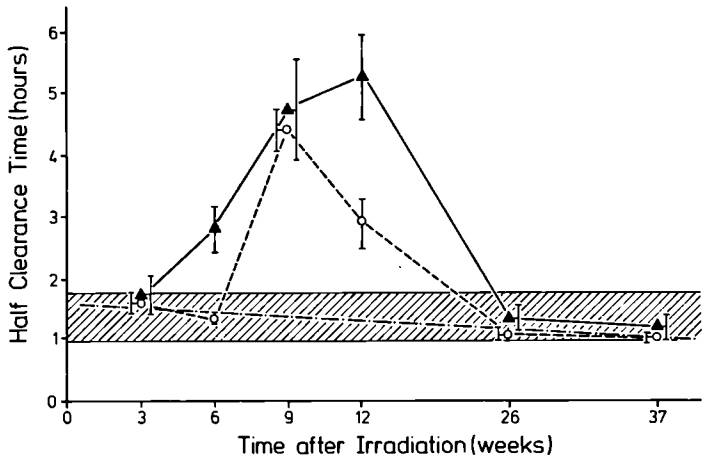

Figure 1: Time-related changes in the half-time of clearance (±SE) of the ^{99m}Tc-colloid from irradiated (▲) and unirradiated (o) pig skin
-·-·-·- indicates best fit to the results for unirradiated skin at 3, 6, 26 and 37 weeks, the hatched area represents the range of clearance values obtained for unirradiated skin.

IV. Objectives for the next reporting period:

Previous studies carried out over the period of this contract have shown phases of development of both atrophy and oedema in the skin of pigs after irradiation. These effects seem likely to produce marked changes in the biomechanical properties of the skin which, in its turn, might influence the physical integrity of the skin. Studies designed to investigate changes in the biomechanical properties of skin after irradiation are now in progress.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Morris, G.M. and Hopewell, J.W. (1988) Changes in the cell kinetics of the pig epidermis after single doses of x-rays. Brit. J. Radiol. 61, 205-211

van den Aardweg, G.J.M.J., Hopewell, J.W. and Simmonds, R.H. (1988) Repair and recovery in the epithelial and vascular conective tisue of pig skin after irradiation. Radioth. Oncol. 11, 73-82

Hopewell, J.W. and van den Aardweg, G.J.M.J. (1988) Radiobiological studies with pig skin (editorial) Int. J. Radiat. Oncol. Biol. Phys. 14, 1047-1050

Hamlet, R. and Hopewell, J.W. (1988) A quantitative assessment of changes in the fibroblast population in the dermis of pig skin after single doses of x-rays. Int. J. Radiat. Biol. 54, 675-682

Morris, G.M., Rezvani, M., Hopewell, J.W., Franke, H. and Loeffler, M. (1988) Epidermal cell kinetics in pig skin. Epithelia, 1, 231-242

Baker, M.R., Bader, D.L. and Hopewell, J.W. (1988) An apparatus for the testing of the mechanical properties of the skin 'in vivo' : its aplication to the assessment of normal and irradiated skin. Bioeng. Skin. 4, 87-103

Title of the project no.: 2

The biological effects of non-uniform irradiation of pig skin

Head(s) of project:

Dr. J.W. Hopewell

Scientific staff:

Dr. G. van den Aardweg
Dr. M. Rezvani
Dr. C. Alcock

I. Objectives of the project:

Concern has been expressed as to the adequacy of existing radiological protection guidelines for the skin. Studies that are being carried out are designed to provide information on the early and late effects of irradiation at different dose-rates, from sources of varying sizes and β-energy. It is hoped that these data will provide an adequate biological basis for an improvement in the present protection guidelines for the skin.

II. Objectives for the reporting period:

Studies have been carried out to establish the effects of dose-rate on the acute radiation response of pig skin to strontium-90 irradiation from a standard 22.5mm diameter source.

III. Progress achieved:

Preliminary studies have been carried out to determine the influence of dose-rate on the acute epithelial response of the skin of pigs to irradiation with strontium-90 plaques 22.5mm in diameter. The dose-rates of the source used were 0.007Gy/min, 0.023Gy/min, 0.048Gy/min and 0.102Gy/min. The results obtained using these low dose-rate sources have been compared with those from a high dose-rate acute exposures at ~3Gy/min.

Following irradiation with the high dose-rate source a steep dose-effect relationship was obtained for the proportion of fields irradiated that developed moist desquamation, i.e. the incidence of desquamation was ~20% after 21Gy rising to ~80% after 33Gy (Figure 1). The dose associated with a 50% incidence of moist desquamation (ED_{50} ± SE) was 27.3 ± 0.5Gy. Irradiations carried out over the period 1979 - 1984 have shown this value to relatively constraint with time, varying from 26.8Gy to 28.11Gy. These values were not significantly different (Table 1).

Table 1: ED_{50} values (±SE) for moist desquamation in pig skin after β-irradiation with a 22.5mm diameter Strontium-90 plaque (3Gy/min)

Irradiation Period	ED_{50} ±SE (Gy)	
1979-1981	27.05 ± 1.12	(4.1%)[a]
1982	26.80 ± 1.25	(4.7%)
1983	27.31 ± 1.01	(3.7%)
1984	28.11 ± 0.91	(3.2%)

a. Percentage error (±SE) on the ED_{50} values

A re-evaluation of the results obtained between 1979 and 1984 has also been used to determine the possible influence of the position of the field on the flank of pigs. The fields were sub-divided according to whether they were on a dorsal, lateral or ventral site. The results of this analysis are given in Figure 1. There was a small site variation from 26.5 ± 0.9Gy for ventral sites to 28.2 ± 0.6Gy for dorsal sites. This difference was not significant ($p>0.1$).

The results obtained with the lower dose-rate sources showed a marked dose-rate effect. The dose-effect curves obtained using the low dose-rate sources were also significantly shallower than those obtained using the high dose-rate source. The preliminary results are shown in Table 2.

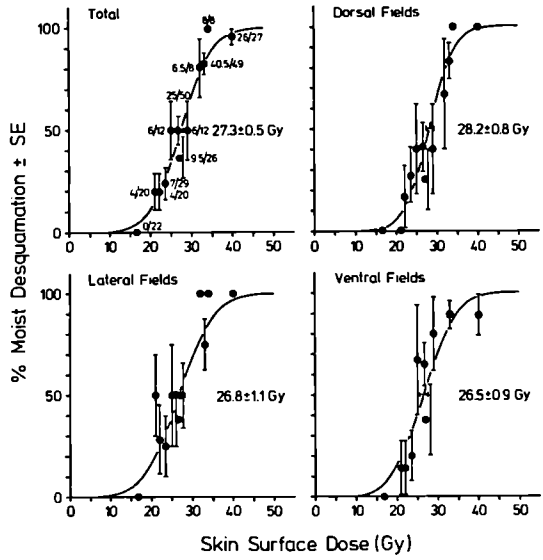

Figure 1: Percentage incidence of moist desquamation after single doses of β-irradiation from a 22.5mm diameter ^{90}Sr-plaque. Fields were pooled or subdivided according to their position on the flank. Small figures represent the number of fields developing moist desquamation against total number irradiated.

With the very low dose-rate source very high doses can be given involving exposure times of >6 days. These long exposure times do not represent a practical problem in radiological protection since regular monitoring of personnel should detect contamination within this time scale.

Table 2:
Influence of dose-rate, from 22.5mm strontium-90 sources on the approximate iso-effect doses for moist desquamation in pig skin

Dose-rate (Gy/min)	Approximately ED_{50}(Gy)	Approximately ED_{20-80}
3	27.3	21 - 33
0.102	36 - 44	<30 - <50
0.048	35 - 40	<35 - <50
0.023	>70	<50 - >70
0.007	>95	<95 - >95

IV. Objectives for the next reporting period:

Late dermal atrophy will be assessed in pig skin irradiated with single doses of strontium-90 β-rays from sources of varying diameter. For a fixed, 22.5mm diameter source, the effects of dose-rate on the severity of late dermal atrophy will be evaluated.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. J. Wells and Dr. M.W. Charles
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Dr. J. Coggle
Department of Radiobiology
Medical College of St. Bartholomew's Hospital
Charterhouse Square
London EC1 6BQ

VI. Publications:

Hopewell, J.W., Barnes, D.W.H., Robbins, M.E.C., Sansom, J.M., Knowles, J.F. and Aardweg van den, G.J.M.J. (1988) The relative biological effectiveness of fractionated doses of fast neutrons ($42MeV_{d\rightarrow Be}$) for normal tissue in the pig. I. Effects on the epidermis and dermal/vascular connective tissues. Brit. J. Radiol. 61, 928-938

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre International
de Radiopathologie
B.P. n° 34
F - 92260 Fontenay-aux-Roses

Contract no.: BI6-C-065-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H. Jammet
Centre International de
Radiopathologie
B.P. n° 34
F - 92260 Fontenay-aux-Roses

Telephone number: 1-46.54.49.29

Title of the research contract:

Non-stochastic effects of irradiation in man: diagnosis, prognosis and treatment of acute radiation injury.

List of projects:

1. Biophysical, biochemical and cytological diagnosis of damage to skin and underlying tissues after accidental exposure. Improvement of therapeutic protocols.
2. Biological indicators of global irradiation.

Title of project no: 1
Biophysical, biochemical and biological indicators of acute local irradiation. Improvement of treatment(s).

Head(s) of project:
Dr R.Gongora

Scientific staff:
B.Perdereau
H.Magdelénat

I. Objectives of the project:
Evaluation of paraclinical, biophysical and biological investigation in the assessment of topology and extent of human acute local irradiation in order to optimize and monitor surgical and/or medical treatment(s).
Evaluation of new therapeutic agents in acute local radiolesions.

II. **Objectives of the reporting period:**
Assessment of topology and extent of vascular damage by vascular scintigraphy, capillaroscopy and thermography.
Assessment of human skin irradiation by biophysical methods: pH metry and evaporimetry.
Evaluation of i.v. and percutaneous administration of radical scavengers (superoxyde dismutase) in the treatment of early and late radiation damage to connective tissues.
Molecular and cellular biology of fibrosis.

III. **Progress achieved:**
Telethermography and capillaroscopy:
From the accumulated experience with the two methods exploring altered vascular flows in irradiated tissues and morphological modifications of ingueal capillaries, we have derived a semiology of skin irradiation. We propose a capillaroscopic grading for the accidental irradiation of the hands (manuscript in preparation) based on the morphological modifications of ingueal capillaries. We have defined the relevant information of telethermographic explorations as a help to the therapeutical decision (manuscript in preparation).

Microwave imaging:
The study of physical parametres being achieved, we have studied the physiological parametres which influence the restitution of the final image provided by the microwave camera.
Among them, the vascular flow was found to be prominent. Temporal variations in volume, or flows (blood flow), alter the response and the

final image. These parametres must be controlled (or can be used for physiological studies). A large part of the work was devoted to the derivation of correcting factors in the computer image processing and to the use of blood flow kinetic measurement for on-line correction of image alterations.

Therapy:
We have initiated a therapeutic protocol of i.v. + percutaneous administration of radical scavengers (SOD, catalase) on early or late radiodermitis following radiotherapy.

Biology of fibrosis:
Measurement of c-myc, Ki67 nuclear antigens and BudR incorporation by flow cytometry has been successfully applied to solid human tissues and cell lines with varying proliferation rates. We intend to apply this methodology to quantify the radiation induced growth stimulation of skin fibroblasts.
For the same purpose, in situ hybridisation of TGF-ß expression has been studied in human cell lines.

IV. **Objectives of the next reporting period:**
- Further evaluation of the therapeutical worth of radical scavengers in the treatment of early and late radiation induced skin lesions.
- Use of flow cytometry to measure the proliferation kinetics of irradiated fibroblasts: ex vivo BudR labeling and *in vivo* BudR labeling. Material: derm fibroblasts from post-radiotherapy surgical samples.
- Measurement of PDGF, TGF-ß and EGF receptors in irradiated fibroblasts. In situ hybridisation of TGF-ß, EGF.R (possibly PDGF.R) in irradiated fibroblasts.

Title of project no: 2
Biological indicators of global irradiation.
Restoration of hematopoietic function after acute global irradiation.

Head(s) of project:
Dr Jammet
H.Magdelénat

Scientific staff:
D.Thierry
O.Rigaud

I. Objectives of the project:
1) Biological evaluation of damage to the hematopoietic system for diagnosis and prognosis of global homogeneous or heterogeneous irradiation in man.
Radiobiology of irradiated bone marrow stroma
Therapeutic protocols for the restoration of the hematopoietic function after irradiation.
In vitro amplification of human hematopoietic stem cells.
2) Biochemical and physiological aspects of DNA repair.

II. Objectives for the reporting period:
1) The role of hematopoietic growth factors in bone marrow restoration has recently been emphasized. More information is needed on:
1. -the effects of in vivo administration of recombinant hematopoietic growth factors in qualitative and quantitative bone marrow restoration;
2. -the role of bone marrow stroma in the restoration of hematopoietic function;
3. -the potential use of in vitro amplification of hematopoietic stem cells in autologous bone marrow transplantation.
2) Evaluation of the genotoxicity to circulating lymphocytes by therapeutical irradiation. Analysis of DNA damage (chromosomal aberration, mutation frequency and repair efficiency) after conventional radiation therapy.
3) Molecular and cellular mechanisms of bone marrow fibrosis.

III. Progress achieved:
1) Bone marrow restoration
The accumulated experience on bone marrow transplantation for therapeutic use and the recent lesson of the Tchernobyl accident have emphasized the risks of allogenic bone marrow transplantation after total body or acute global irradiation. Autologous bone marrow transplantation tends to replace allogenous transplantation, but is still limited to

particular situations where residual autologous stem cells are available or can be amplified in vivo or in vitro. Hematopoietic growth factors are currently evaluated for reducing the lead time of hematopoietic recovery and in the prospect of in vitro amplification of hematopoietic stem cells and stromal cells.
Due to the impossibility of irradiation of healthy human beings, all investigations were carried out within therapeutical protocols (in vivo or ex vivo) or in vitro.

1.1 Allogenic bone marrow transplantation
It remains the only alternative in most accidental situations of lethal bone marrow irradiation. Besides the problem of immunotolerance, the capacity of the irradiated stroma to sustain hematopoietic restoration is determinant in hematopoeitic recovery.
We have addressed this problem by the study of the cooperative role of stromal and hematopoietic cells in long term bone marrow culture in vitro and ex vivo.
In collaboration with the Service d'Hématologie (Dr E.Gluckman), Hôpital St.-Louis, Paris, long term (4 to 10 weeks) bone marrow cultures (LBTMC) could be established for 21 patients suffering from primitive aplasia. Serial subcultures of GM progenitors (CFU-C) indicated a quantitative defect of hematopoiesis whereas immunochemical characterization showed no qualitative modification of the stromal cell types.

1.2 Autologous bone marrow transplantation
LTBMC has been studied before and after transplantation of purged autologous bone marrow with or without GM CSF in children treated for neuroblastoma (96 LTBMC). At diagnosis, LTBMC of these patients behave as healthy donors regarding the yield of progenitors and the stromal cell growth. After total body irradiation (12 Gy), the damage to the bone marrow microenvironment was reflected by stromal cell growth in LTBMC. The administration of GM-CSF to the patients after transplantation did not result in obvious improvement of LTBMC, nor in measurable circulating hematopoietic progenitors in the peripheral blood.

1.3 Hematopoietic stem cell amplification in vitro
The results of LTBMC in pathological situations indicate its interest as a model integrating hematopoietic- stroma interactions, as a test for monitoring therapy or predicting hematopoietic function recovery, but stress the limits of its applicability to limited aplasia. Three attempts to increase the yield of hematopoietic progenitors in LTBMC have been studied:

1. -the use of defined culture media;
2. -three-dimensional culture or subculture in agar or on cellulose acetate filtres
3. -preliminary use of recombinant growth factors (GM CSF, G.CSF, etc.)

None of these conditions were superior to the reference method of Dexter.

2) Genotoxic effects of radiation therapy on circulatory lymphocytes.

We have compared the genotoxic effects of conventional radiotherapy (60 Gy) and chemotherapy (CAF) on the circulating lymphocytes of breast cancer patients. Four end points were tested at different phases of treatment:

1. -chomosome aberrations in circulating lymphocytes
2. -chromosome aberration yield after an in vitro test - irradiation (4 Gy);
3. -DNA strand breaks and repair after in vitro test irradiation;
4. -mutation frequency in HGPRT locus

No significant difference between cancer patients before treatment and healthy donors was observed for any of the end points tested.

Local radiotherapy of breast tumors induced a frequency of chromosome aberrations (dicentrics or fragments) in circulatory lymphocytes equivalent to about 2 Gy for total body irradiation. DNA repair efficiency of in vitro test irradiation was significantly, although to a low and variable degree, decreased after radiotherapy. There was, however, no significant increase in the additional yield of aberrations by in vitro test irradiation, compared to not previously irradiated lymphocytes.

Cytotoxic chemotherapy (anticancer drugs) did not induce elevated frequency of chromosome aberrations in treated patients, nor alter the repair efficiency of lymphocytes.

Mutations at the HPRT locus were systematically increased after radiotherapy, not after chemotherapy. However, the augmented frequency was still within the range of individual variations observed in healthy donors. The conclusion of this study was the great intra and inter individual variability in human lymphocyte response to genotoxic agents which limits its use as a target cell for the prediction of individual radiosensitivity.

Biology of bone marrow fibrosis:

The role of megakaryocytes and platelet-derived growth factors was investigated. Rabbit polyclonal antibodies have been raised from a c-terminal synthetic peptide of TGF-ß, which cross-react with and precipitate active TGF-ß. However, the ELISA assay of TGF-ß in platelets was not satisfactory due to matrix effects from bulk proteins.

A radioreceptor assay is currently investigated.
PDGF has been assayed in platelets from patients with bone marrow fibrosis (myeloid splenomegaly).

IV: **Objectives for the next reporting period**
1) To study in vitro and in mice the role of hematopoietic growth factors in bone marrow restoration, with special focus on stem cell recruiting factors (IL1) since radioprotective effects of this intraleukine have been described.
2)- To study the physiological parametres influencing the lymphocyte response.
- To study the role of repair mechanisms in the yield of chromosomal aberrations and mutation frequency.
- To study the critical genotoxic events, at the molecular level, leading to cell death.
3) To study the role of platelet derived growth factors in fibrosis of hematopoietic tissue. A radioreceptor assay of TGF-ß and TGF-ß receptors will be developed. PDGF, TGF-ß and EGF-like assays in platelets of various pathological situations involving medullary fibrosis will be carried out. In situ hybridisation of the mRNA of these factors will be attempted in bone marrow megakaryocytes.

V: **Other research group(s) collaborating actively on this project [name(s) and address(es)]:**
Service d'Hématologie (Dr E.Gluckman), Hôpital St.-Louis, Paris
Laboratoire de Radiopathologie (Dr H.Magdelénat), Institut Curie, Paris
DPS/SPE (Dr M.T.Doloy), Fontenay-aux-Roses, France
Laboratoire de Radiobiologie, CNRS n° (Dr E.Moustacchi), Institut Curie, Paris
Service de Pédiatrie oncologique (Dr Zucker), Institut Curie, Paris
Laboratoire de Physiologie et Pharmacologie de l'Endothélium (Pr Tobelem), Hôpital Lariboisière, Paris

VI: **Publications:**
D.THIERRY, F.VARRIN, M.HARDY, A.DEVERGIE, H.MAGDELENAT, E.GLUCKMAN. Three-dimensional outgrowth of normal human bone marrow cells. American Society of Hematology, Seattle Symposium. 16, 1988.

F.VARRIN, D.THIERRY, M.BENBUNAN, A.DEVERGIE, H.MAGDELENAT, E.GLUCKMAN. Long term bone marrow cultures in the treatment of aplastic anemia. European Cooperative Group for Bone Marrow Transplantation, Poster, p.62, Lecture and Publication in Bone Marrow Transplantation, 3 sup.1, 242, 1988.

K.BEN MAHREZ, D.THIERRY, I.SOROKINE, J.DANIEL, A.BRACONE, M.KOHIYAMA Détection d'anticorps anti-protéine d'oncogènes circulant dans le sérum de malades atteints de cancer. 6ème Ecole franco-africaine de biologie molécualire, ed.S.Benhamida, pp.224-226, 1988.

D.THIERY, P.VALIDIRE, M.HARDY, H.MAGDELENAT, J.M.ZUCKER. Long term bone marrow culture in metastatic neuroblastoma. In press Eur J Cancer and Clin Oncol, 1988.

D.THIERRY, J.MICHON, M.HARDY, P.VALIDIRE, H.MAGDELENAT, J.M.ZUCKER. Long term bone marrow treatment of disseminated neuroblastoma. Submitted to European Cooperative Group for Bone Marrow Transplantation, EBTM 89.

G.GUEDENEY, O.RIGAUD, I.DURANTON, J.L.MALARBET, M.T.DOLOY and H.MAGDELENAT. Chromosomal aberrations and DNA repair ability of in vitro irradiated white blood cells of monkeys previously exposed to total body irradiation (in press)

O.RIGAUD and H.MAGDELENAT. Fluorimetric analysis of DNA strand breakage and repair kinetics. Application to radiotoxicology. In "New Trends in Genetic Risk Assessment", ed.G.Jolles end A.Cordier, Academic Press, New York, in press.

R.GONGORA. Irradiations accidentelles. Revista di Medicina del Lavoro ed Igiene Industriale. Idelson ed. (Naples), pp.99-153

TITLE OF THE PROJECT N° : B 160065 F

Modifications cellulaires et biochimiques du sang après irradiation corporelle totale.

HEAD(S) OF PROJECT :

Pr. J.DUTREIX
Pr. J.M.COSSET

SCIENTIFIC STAFF :

- Laboratoire de radiobiologie cellulaire
Unité INSERM 247 - (Drs E.MALAISE, M.GUICHARD, T.GIRINSKY)

- Laboratoire de Biologie clinique - Institut Gustave-Roussy
(Pr. C.BOHUON, Dr E.COMOY).

- Laboratoire d'Hématologie de l'Institut Gustave-Roussy
(Dr C.BAYLE).

I. OBJECTIVES OF THE PROJECT

1/ Déterminer les possibilités de typage HLA après irradiation totale à 10 Gy (en dose unique).

2/ Etude des variations des granulocytes et des lymphocytes après irradiation totale à doses et fractionnement variables.

3/ Etude de l'évolution de divers paramètres biologiques après irradiation totale à doses et fractionnement variables.

Les points 2 et 3 cherchent à déterminer dans quelle mesure certains paramètres peuvent être utilisés comme "dosimètres biologiques", susceptibles d'évaluer rétrospectivement la dose reçue en cas d'accident d'irradiation.

II. OBJECTIVES FOR THE REPORTING PERIOD

1/ Rapport des typages HLA pour 5 patients prélevés 24 heures après une irradiation totale de 10 Gy.

2/ Etude des variations hématologiques (granulocytes et lymphocytes) chez :
- . 8 patients après 10 Gy d'irradiation totale
- . 10 patients ayant reçu une irradiation totale fractionnée de 12 Gy en 6 séances et 3 jours
- . 5 patients ayant reçu une irradiation totale hyperfractionnée de 13,2 Gy en 11 séances et 4 jours.

3/ Etude chez ces mêmes patients des variations
- a) du cortisol plasmatique et de l'ACTH
- b) de la Nor adrénaline et de l'adrénaline
- c) des hormones thyroïdiennes et de la TSH
- d) de l'amylasémie (+ études complémentaires chez 15 patients ayant reçu une irradiation localisée de la parotide et chez 15 patients ayant reçu une irradiation pancréatique).
- e) des gaz expirés (éthane, pentane) reflet théorique de la création de radicaux libres.

III. PROGRESS ACHIEVED

1/ Typage HLA, 24 heures après 10 Gy d'irradiation totale ; a été possible 3 fois (sur 5 patients étudiés).

2/ Variations hématologiques :
- a) granulocytes :
  Un pic granulocytaire est retrouvé de façon constante 4 à 8 heures après le début d'une irradiation totale de 10 Gy en 4 heures. Les résultats sont moins spectaculaires et moins constants après la première séance (1,35 ou 2 Gy) des irradiations fractionnées.
- b) lymphocytes : la baisse est rapide et constante après 10 Gy délivrés en 4 heures, le taux est de 25 à 50 % de la valeur initiale 8 heures plus tard, 10 à 30% 24 heures plus tard.

3/ Variations des paramètres biologiques.
- a) Cortisol : élévation constante à la fin de l'irradiation de 10 Gy en 4 heures et 4 heures plus tard. Les résultats sont moins constants et moins marqués après les irradiations de 1,35 et 2 Gy. (corrélation avec le pic granulocytaire ?).
- b) Nor adrénaline et adrénaline :
  Variations mineures, non significatives.

c) Hormones thyroïdiennes - TSH
Variations non significatives

d) Amylasémie
Pic d'amylasémie constant après irradiation totale : le pic parait dose-dépendant mais avec des variations individuelles marquées.
Chez les 15 malades ayant reçu une irradiation localisée de l'anneau de Waldeyer à 2 Gy : le pic d'amylasémie est constant et la valeur moyenne du pic est similaire à celle observée chez les malades irradiés totalement à la même dose.
Chez les 25 malades ayant reçu une irradiation pancréatique de 2 Gy ; aucune augmentation de l'amylasémie n'a été détectée.
L'origine salivaire de l'hyperamylasémie après irradiation totale parait donc ne pas faire de doute.

e) Les gaz expirés ; les problèmes techniques liés à la difficile extrapolation à l'homme d'une procédure mise au point chez de petits animaux expliquent peut être les résultats négatifs (pas d'augmentation décelables de l'éthane et du pentane) chez nos patients.

IV. OBJECTIVES FOR THE NEXT REPORTING PERIOD

1/ Poursuite des typages HLA, en rajoutant un prélèvement à 6 H après irradiation totale.

2/ Poursuite de l'étude des variations des granulocytes et lymphocytes pour inclure un nombre plus important de patients.

3/ Paramètres biologiques.
Poursuite des dosages de cortisol pour tenter de préciser l'importance et la chronologie du pic et pour affiner la corrélation probable avec le pic granulocytaire.

Poursuite des dosages d'ACTH, de Nor adrénaline, d'Adrénaline, des hormones thyroïdiennes et de TSH pour confirmer leur non réponse à l'irradiation avec un plus grand nombre de patients.

Affinement des études sur l'amylasémie : poursuite des dosages après irradiation totale, mais en parallèle recherche d'un seuil grâce à des séances de 0,5 et 1 Gy effectuées dans le seul anneau de Waldeyer. Précision de la relation dose reçue-amylasémie.

Poursuite des études des gaz expirés en modifiant les techniques de prélèvement.

Mise au point du dosage et études des variations de l'interleukine 1 et du INF alpha après irradiation totale.

V. OTHER RESEARCH GROUP(S) COLLABORATING ACTIVELY ON THIS PROJECT (NAMES AND ADRESSES :

Dr RAFFOUX - Laboratoire de greffe de moëlle France-Transplant.
Hôpital Saint Louis. PARIS

Dr DOLOY - Laboratoire de Physiopathologie Expérimentale
Centre d'Etudes Nucléaires - FONTENAY AUX ROSES

VI. PUBLICATIONS

1. DUTREIX J., GIRINSKY T., COSSET JM., BERNARD A., PICO J., BAUME D., BAYLE C., BENK V.
   Blood cell kinetics and total body irradiation.
   Radiotherapy and Oncology, 1987, 9, 119-129

2. DUTREIX J., GIRINSKY T., BENK V., COSSET JM., BERNARD A., PICO J., BAUME D.,
   Evolution du taux des lymphocytes au cours de l'irradiation corporelle totale hyperfractionnée.
   In Radiophysique, Recueil des communications du XXVème congrès de la Société Française des physiciens d'hôpital.
   Toulouse 5-7 juin 1986, 463-469

3. GIRINSKY T., COSET JM., PICO J., BAUME D., BERNARD A., DUTREIX J., MALAISE E.
   Peripheral blood lymphocyte subsets after low-dose (1.2, 1.35 Gy) total body irradiation.
   Proceedings of the 14th European cooperative group for bone marrow transplantation.
   Chamonix 10-13 avril 1988. Bone marrow transplantation, 1988, 3, suppl. 1, p.303

4. DUTREIX J., GIRINSKY T., HUBERT D., SOCIE G., COSSET JM.
   Early blood cell kinetics after total body irradiation.
   Biological and clinical significance.
   Radiation Research (in press)

5. HENNEQUIN C., COSSET JM., CAILLEUX P.E., GIRINSKY T., GANEM G., HUBERT D., BOHUON C., DUTREIX J.
   Soumis au Bulletin du Cancer
   L'amylasémie : un marqueur biologique des irradiations accidentelles ?
   Revue de la littérature et résultats préliminaires obtenus à l'Institut Gustave Roussy.

Title of the project no.:

BIOPHYSICAL METHODS
FOR IMPROVING INTERNAL CONTAMINATION DIAGNOSIS

Head(s) of project:

LEMAIRE G.
FRANCK D.

Scientific staff:

MORONI J.P.
PELLERIN P.
REMY M.L.

I. Objectives of the project:

Perfecting of automatic and computerized procedures to assess internal contamination of people in case of a nuclear accident, and of making active phantoms needed for calibrating the measuring devices.

The main objective is to assess the biological damage by measuring internal activity by means of mobile units working on the spot for very large population screening.

II. Objectives for the reporting period:

Perfecting of a method enabling internal gamma contamination assessment in less than five minutes by means of mobile gamma spectrometer units linked with a computer.

III. Progress achieved:

1 - Measurements

A method of measuring internal contamination of people on the spot has been carried out. It enables the assessment of internal gamma activity in less than five minutes on 4, 12 or 32 people simultaneously, whatever the nuclide may be.

Measurement thresholds depend upon two main parameters :

- the nature of the radionuclide

- the background level

For Iodine and Cesium radionuclides, these thresholds are respectively 40 and 1000 Bq for the ICRP standard man and for natural background.

This procedure authorizes a quick triage of a very large population and doing so to achieve any treatment if needed.

2 - Phantoms

Phantoms made of foam, achieved by mixing silicon and powder of different densities with a catalyser, have been carried out.

All the tests which have been already carried out clearly demonstrate that this new technology of designing phantoms or calibrating sources allows the making of different density radioactive sources (densities in the range 0.2-1) adapted to the metrological goal.

IV. OBJECTIVES FOR THE NEXT REPORTING PERIOD

- Automatic recording of relevant people parameters

- Use of a P.C. to improve the capacity and rapidity of data processing

- Use of analogic-numeric devices to get rid of any spectrometer

V. PUBLICATIONS

PELLERIN P.
LEMAIRE G.
FRANCK D.

SCPRI'S ANTHROPO AND ENVIRONMENTAL MOBILE SPECTROMETRIC UNITS

To be published in :

Fourth International Symposium
MALVERN, ENGLAND - 4-9 June 1989

RADIATION PROTECTION PROGRAMME

Progress Report

1986

Contractor: Contract no.: BI6-C-071-B

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Rue Charles Lemaire, 1
B - 1160 Bruxelles

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M. Janowski
Département de Radiobiologie
CEN/SCK
Boeretang 200
B - 2400 Mol

Telephone number: 014-31.18.01

Title of the research contract:

Late somatic effects of radiation in mammals.

List of projects:

1. Late effects of an in utero irradiation on the central nervous system.
2. Early and late radiation damage to the hemopoietic and immune system of new-born animals.
3. Effect of fractionation of gamma rays and neutrons on cancer induction and promotion in mouse liver.
4. The effect of age on tumour induction by radiation alone or combined with a chemical carcinogen.

Title of the project no.: [1]

Late effects of irradiation in the developing central nervous system

Head(s) of project:

J.R. Maisin, G.B. Gerber, H. Reyners

Scientific staff:

I. Objectives of the project:

To reveal and study the mechanisms of immature cell radiosensitivity (mostly in fetal but also in young adult rats) using a combined morphological and biochemical approach. Also of importance is to determine the relationship between effects and dose or radiation quality using a spectrum of irradiation protocoles.

II. Objectives for the reporting period:

A. Morphological assays

1. Short term (3 months) studies after fetal irradiation using gamma or neutron fetal irradiation.
2. Long term studies (15 months & 24 months) after X and neutron fetal irradiation.

B. Biochemical assays

1. Determination of biogenic amines after 1.5 Gy.
2. Determination of opiate and histamine receptors after 1 Gy.

III. Progress achieved:

A. Morphology

1. Short term studies after fetal irradiation at day 15 of pregnancy

a) NEUTRON IRRADIATIONS : Covariance analysis of the decrease of the cingulum (a corpus callosum tributary) volume in 73 rats after 5 increasing doses (up to 10 cGy) of 500 kV neutrons, using the rat brain weight as covariate, highlights the high significancy of this tendency. In such conditions, it appears that white matter development is selectively inhibited by the irradiation.
Limiting the preceding experimental setup to 2 doses only (0 and 2.5 cGy), variance analyses still reveal nearly significant atrophy ($P < 0.075$) of the cingulum in spite of the large coefficient of variation bound to this parameter ; however, the decrease in brain weight is highly significant ($P < 0.001$).

b) LOW DOSE RATE GAMMA IRRADIATION : Variance analysis revealed highly significant ($P < 0.001$) decrease of the brain weight in 15 rats treated with 20 cGy ^{60}Co gamma rays delivered at 4 cGy per hour.

2. Long term studies after fetal irradiation (at day 15 of pregnancy)

a) 15 MONTHS AFTER 600 kV NEUTRON : Covariance analysis of brain weights with body weights as covariates was carried out in 58 fifteen month old rats treated with 0 to 10 cGy of 600 kV neutrons. Brain weights were found significantly ($P = 0.03$) lowered down to the 2.5 cGy level although body weights were not concerned after such low dose.

b) 24 MONTHS AFTER X-RAYS : Nearly significant ($P = 0.075$) decrease of the brain weight was observed in old rats treated with up to 10 cGy X-rays (22 controls versus 9 animals treated with 5 cGy and 8 with 10 cGy.

3. Adult studies

X-irradiation of the brain of young adult rats were performed in order to test the behaviour of the residual embryonary cell in this organ. The study reveals that, 1 month after this treatment, microglial elements decrease according to the increasing dose of X-rays delivered to the brain. However, after doses higher than 20 Gy, this tendency reverses and microglial populations rise again : we assume that reproductive death of these putative precursor elements is inhibited after high radiation doses by a sort of G2 delay prolongation.

B. Biochemistry

1. BIOGENIC AMINES : Rat brain was exposed in utero to 1.5 Gy of X-rays on day 10, 12 and 15 of pregnancy and the offspring was studied at an age of 1-5 Months. A number of experiments were also carried out after 2.5 MV neutron exposures. The rather high X-ray dose of 1.5 Gy was chosen since the changes found after lower doses were not always consistent, but of course, this dose caused considerable brain damage and brought reduction

of up to 64 % in brain weight when exposure was carried out on day 15 of pregnancy. Weight loss was somewhat smaller when exposure took place earlier in pregnancy.

Two general reactions were observed : a) a reduction of the total amount of biogenic amine per brain structure due to the atrophy of this structure ; however, the concentration of the amine does not change with respect to control level. This is the case of dopamine in hypothalamus, cortex and striatum and also of epinephrine in the latter structure. b) an increase in concentration with a constant content or a reduction per structure. This was found, for example, for epinephrine in hypothalamus, medulla, cerebellum and cortex. These results seem to indicate that biogenic amine level is maintained even in the presence of gross structural abnormalities.

2. RECEPTORS : Three types of receptors were studied after exposure to 16 Gy at different times of pregnancy. The Kd and Bmax were evaluated by non-linear regression analysis or by a linear Scatchard transformation. Flunitrazepam receptors show no change with age or after irradiation in cortex and cerebellum when evaluated by non-linear regression whereas Kd and Bmax seem to decrease with age but also in 1 month old irradiated pups in Scatchard plots. This is possibly due to the presence of a small component with different binding characteristics. In pyrilamine binding receptors (H1 histamine receptors), both Kd and Bmax increase with age. Irradiation seems to retard this increase in the frontal cortex at an age of 3 months. However, Bmax significantly increases 6 months after exposure at 15 days of pregnancy (no changes were found in hippocampus). Fentanyl binding (opiate receptors) show an increase of Bmax in the 3 month old cortex irradiated on day 10 after conception. Again, no changes were found in hippocampus.

IV. Objectives for the next reporting period:

A. Morphology

1. Morphometric analysis of the white matter and automatic image analysis to search for the origin of the atrophy.
2. Short term effects (in 3 month old rats) of very low dose rate fetal X-irradiation.
3. Histoautoradiographic analysis of the regeneration of residual embryonary cells after X-irradiation of the adult brain.

B. Biochemistry

1. Autoradiography of benzodiazepine and opiate receptors.
2. Analysis of aminoacids in different brain areas after 1.5 Gy.
3. Analysis of cholinergic receptors.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

H. Liskien (Euratom, CBNM at Geel) and F. Poortmans (SCK/CEN Neutron Physics at Mol) were responsible for the neutron irradiation and dosimetry. G. Konermann (Univ. Freiburg, FRG) provides assessment for image analysis.

VI. Publications:

H. Reyners : Les effets tardifs des radiations ionisantes dans le cerveau du rat. Doctoral thesis presented at the Catholic University of Louvain-la-Neuve.

H. Reyners : Ultrastructural and cell kinetic changes in glial cells after irradiation. Proc. 21st Meeting European Society for Radiation Biology, Tel Aviv, Oct. 88. VCH Publ. Weinheim, FRG.

H. Reyners, E. Gianfelici de Reyners, J.R. Maisin : Intermitotic cell death of immature glial cells after X-irradiation of the adult rat brain. Accepted by the Intern. J. Radiat. Biol.

H.S. Reinhold, J.W. Hopewell, W. Calvo, A. Keyeux, H. Reyners : Effects of radiation on the normal vasculature. 7th ESTRO Meeting, Den Haag, September 88.

Title of the project no.: 2

Early and late radiation damage to the hemopoietic and immune system of new-born animals

Head(s) of project:

L. de Saint-Georges

Scientific staff:

L. de Saint-Georges, M. Janowski, R. Hooghe, G. Gerber

I. Objectives of the project:

The working hypothesis is that the radiosensitivity of the haemopoietic system (and the immune system in particular) is different in adult and in developing animals. Short- and long-term consequences of prenatal and neonatal irradiation are therefore studied.

II. Objectives for the reporting period:

1. Study of the behaviour of mouse and rat stem cells during the first year of life after post-natal irradiation : reticulocyte response after low pressure stimulation.
2. The effect of prenatal and early post-natal irradiation of rats and mice on the immune system.

III. Progress achieved:

Objective 1

1. Methodology

BALB/c and C57BL mice and Wistar rats, were X-irradiated at the age of 6 days and 3 months. Groups of 10-11 animals served as nonirradiated controls or were submitted to either single doses of 1.1, 2.0, 3.1 Gy or a fractionated dose of 1.1 + 2.0 Gy (at a 3-day interval). The reticulocyte response was determined 0, 14, 30 and 90 days after a 3-day low pressure (350 mbar) stimulation.

2. Results

The present, and still partial results are summarized in Table I.

TABLE I. RETICULOCYTE RESPONSE OF X-IRRADIATED MICE AND RATS TO LOW PRESSURE STIMULATION (reticulocytes/100 red cells)

Age	6 days				3 months			
Recovery (d)	0	14	30	90	0	14	30	90
Balb/c								
control	21.90	5.09	4.91	2.61	3.39	2.31	2.88	2.74
1.1 Gy	22.02	6.67	NA*	NA	1.65	1.53	3.49	NA
2.0 Gy	NA	NA	NA	NA	NA	2.91	2.93	NA
3.1 Gy	7.63	6.96	2.86	2.35	0.07	5.96	1.71	0.83
1.1+2.0 Gy	8.29	5.41	4.80	4.14	1.34	4.94	3.28	2.45
C57BL								
control	20.37	13.93	2.20	0.88	2.09	1.30	1.29	0.76
1.1 Gy	24.93	NA	NA	NA	1.37	3.09	2.32	NA
2.0 Gy	NA	NA	NA	NA	0.58	2.32	3.13	NA
3.1 Gy	6.87	8.72	7.48	1.64	0.04	2.58	2.19	0.92
1.1+2.0 Gy	10.11	11.37	4.46	2.74	0.07	2.20	1.75	0.84
Rats								
control	45.62	18.38	10.79	3.81	4.03	3.07	2.29	2.19
1.1 Gy	NA	NA	NA	NA	1.11	2.69	NA	NA
2.0 Gy	NA	NA	NA	NA	NA	NA	NA	NA
3.1 Gy	19.41	35.12	14.60	3.11	0.73	3.45	NA	NA
1.1+2.0 Gy	16.35	20.64	9.22	2.72	1.04	3.76	2.32	1.71

* NA : result non yet available.

In adult mice and rats, irradiation provokes an immediate decrease of the ability to respond to stimulation. A single dose is more efficient than a fractionated dose. The recovery passes through a phase of exagerated response, followed at least in BALB/c mice by a long-term depression for the highest, single dose (3.1 Gy). In neonates, irradiation provokes an immediate (for the lowest dose) or delayed (for the higher doses) increase of the response ability. Later recovery is better, and even more exagerated, after a fractionated dose. In contrast with adults, there is no long-term depression effect.

3. Discussion

The results can be interpreted in terms of a preferential stimulation of erythropoiesis in irradiated neonates compared to adults. One can also consider that damage to neonatal stromal cells, which are more radiosensitive than those of adults, could indirectly exert a stimulation effect on the hemopoietic stem cells, accounting for the differences of reaction between neonates and adults.

Objective 2

1. Methodology

Wistar rats and A/J mice were irradiated (0.25 - 2 Gy) at given times after conception (5-20 days) or after birth (2-8 days). The mouse study has already been reported and published. The data on rats have now been analyzed. The end-points were the histology of the spleen, the serum immunoglobulin levels, and the response to T-dependent or T-independent antigens.

2. Results

At the age of 8 weeks, the histology of the spleen was normal, and so was the distribution of B and T lymphocytes. The serum immunoglobulin levels were not significantly altered, even when the different isotypes were considered. At the age of 10 weeks, rats were immunized with a T-dependent or a T-independent dinitrophenylated-carrier antigen. Normal levels of specific antibodies were generated in all groups of animals injected with the T-independent antigen. The T-dependent response, in contrast, was higher in animals irradiated between day 6 and day 20 of gestation (but not in rats irradiated early after birth). This increase, however, was significant only for the IgM and IgG1 responses of some irradiated groups. Thus no medium-term immunodeficiency could be documented with the methods used. The alteration in a T-dependent response, however, points to a radiosensitive T regulatory mechanism.

3. Discussion

In conclusion, whole-body irradiation (up to 2 Gy) during the development of the haematopoietic system does not result in severe long-term impairment of immune responses. Data from the literature and from our own studies indicate, however, that some long-term effects of irradiation on the immune system can be demonstrated. Idiotypic analysis will be useful in this respect.

IV. Objectives for the next reporting period:

Objective 1

Study of the long term fate of haematopoietic stem cells : transplantation, in lethally irradiated neonatal and adult mice, of homologous bone marrow cells with chromosome marker and test for the reappearance of the original host cells. The first results are presently being analysed.

Objective 2

Idiotypic studies will be done on rat sera. After irradiation, we will also monitor the levels of several growth factors.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Experimental immunology UCL, Brussels (Dr Bazin)
TNO Rijswijk (Prof. van Bekkum)
KFA Jülich (Dr von Wangenheim)
Universität Ulm (Dr Nothdurft)
Animal Physiology, ULB, Brussels (Dr Urbain)
Ludwig Institute for Cancer Research, Brussels (Dr Van Snick)
Université de Liège (Dr Boniver)

VI. Publications:

R. Hooghe, J.R. Maisin, F. Vander Plaetse, J. Urbain, G. Urbain-Van Santen : The effect of prenatal or early post-natal irradiation on the production of anti-arsonate antibodies and cross-reactive idiotypes. Int. J. Radiat. Biol., 53 (1988) 153-157.

R. Hooghe : The immune system : a sensitive indicator of radiation damage? Terrestrial space radiation and its biological effects. P.D. McCormack, C.E. Swenberg and H. Bucker, Editors, Plenum Press, N.Y., 1988 (in press).

B. Platteau, H. Bazin, M. Janowski, R. Hooghe : Failure to detect immune deficiency after prenatal or early postnatal irradiation in the rat. Int. J. Radiat. Biol., 1988 (in press).

Title of the project no.: 3

Effect of fractionation of gamma rays and neutrons on cancer induction and promotion in mouse liver.

Head(s) of project:

J.R. Maisin

Scientific staff:

M. Janowski, L. de Saint-Georges, J. Vankerkom, M. Lambiet-Collier, G. Mattelin

I. Objectives of the project:

1. to study the effects of X-rays alone or combined with carbon tetrachloride (CCl_4) on induction and promotion of cancer in adult mouse liver ;
2. to carry on the analysis of the final results of the study performed during the period 1980-1984 on the relative effectiveness of a single or fractionated whole body gamma or 50 MeV neutron exposure with respect to survival and cancer induction in BALB/c and C57BL mice.

II. Objectives for the reporting period:

1. to follow the lifespan of mice exposed locally on the upper part of the abdomen, with increasing doses of X-rays, treated with a single subcutaneous injection of CCl_4 or exposed to X-irradiation following or preceeding CCl_4 treatment ; to perform autopsy and histological analysis of tissue samples of dead mice ;
2. to finalize the analysis of the results of the study performed during the period 1980-1984 on the relative effectiveness of a single or fractionated whole-body gamma or 50 MeV neutron exposure with respect to survival and cancer induction in BALB/c and C57BL mice.

III. Progress achieved:

A. Effects of X-rays alone or combined with CCl_4 on induction and promotion of cancer in adult mouse liver

1. Methodology

The following 5 groups of mice were followed :

- Three control groups :
  1. normal controls (500 mice)
  2. control mice exposed to 0.5, 1, 2, 4 or 6 Gy of X-rays (285 mice)
  3. control mice receiving subcutaneously 0.1 ml of a solution of 40 % CCl_4 (100 mice)
- Two treated groups :
  1. X-irradiation following CCl_4 treatment : Mice were treated with 0.1 ml of a solution of 40 % CCl_4 and exposed 69 h later to 0.5, 1, 2, 4 or 6 Gy of X-rays (289 mice)
  2. X-irradiation preceeding CCl_4 treatment : Mice were exposed to 0.5, 1, 2, 4 or 6 Gy of X-rays and treated 3 months later with a subcutaneous injection of CCl_4 (406 mice).

2. Results

The experiments are still in progress. Most of the controls and the treated mice were dead on 1.1.1989. Autopsy and histological analysis of tissue samples of 80 % of all control and treated mice were performed. It was confirmed that in both controls (normal controls excepted) and treated mice, the most important causes of death were extrathymic lymphoma and liver tumor (adenocarcinoma, sarcoma, adenoma and angioma). No statistical significant differences were actually observed between the irradiated control group and the two groups of treated mice for the incidence of nonthymic lymphomas. The percentage of liver tumors was increased in the X-irradiated control group and in the two treated groups (X-irradiation following CCl_4 treatment and X-irradiation preceeding CCl_4 treatment) compared with the normal control group. A significant increase in liver tumors was observed in the group of mice exposed to 6 Gy of X-rays followed with CCl_4 treatment compared to the group of mice exposed to 6 Gy only. .

3. Discussion

The results obtained so far seem to demonstrate that CCl_4 given before an X-ray exposure seems not to increase the incidence of liver cancer in C57BL mice compared with X-irradiated control group. The results obtained for mice promoted with CCl_4 three months after X-irradiation show after an exposure to high doses of X-rays (6 Gy), a significant increase of liver tumor as compared with X-irradiated control mice and that no time lag is needed for promotion.

B. Relative effectiveness of a single or fractionated whole-body gamma ray or 50 MeV neutron exposure with respect to survival and cancer induction

The analysis of the final results of the study performed during the period 1980-1984 on the relative effectiveness of a single or fractionated whole-body gamma or 50 MeV neutron exposure with respect to survival and cancer induction in BALB/c and C57BL mice is now complete and the results were published in Radiation Research.

IV. Objectives for the next reporting period:

To carry on the analysis of the final results of mice exposed to X-irradiation following or preceeding CCl_4 treatment.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

BCMN, B-2440 Geel

VI. Publications:

J.R. Maisin, A. Wambersie, G.B. Gerber, G. Mattelin, M. Lambiet-Collier, B. De Coster, J. Gueulette : Life shortening and disease incidence in C57BL mice after single and fractionated gamma and high-energy neutron exposure. Radiation Research 113 (1988) 300-317.

J.R. Maisin : Acute radiation syndromes in man. Terrestrial Space Radiation and its Biological Effects, 154 (1988) 445-463.

J.R. Maisin : Life shortening and causes of death in experimental animals following whole-body exposure to ionizing radiation. Terrestrial Space Radiation and its Biological Effects, 154 (1988) 423-444.

Title of the project no.: 4

The effect of age on liver tumour induction by radiation alone or combined with a chemical carcinogen

Head(s) of project:

J.R. Maisin

Scientific staff:

L. de Saint-Georges, M. Janowski, J. Vankerkom, H. Liskien, M. Lambiet-Collier, G. Mattelin

I. Objectives of the project:

1. to study the influence of age on tumour induction in mice by X- and neutron irradiation
2. to study to which extent small doses of X- and neutron irradiation could potentiate the effects of diethylnitrosamine (DEN) on the induction of liver tumour.

II. Objectives for the reporting period:

1. To study to which extent small doses of X-irradiation could potentiate the effects of DEN on the induction of liver tumour.
2. To expose groups of mice to 3.1 MeV (0.12, 0.25 and 1 Gy) neutrons alone or preceeding or following DEN treatment to study long term survival and tumour incidence in the liver.

III. Progress achieved:

1. Methodology

Mice treated with X-rays :

Ten mice of the following groups : mice treated with DEN alone ; mice treated with X-rays alone ; mice treated with DEN + X-rays ; mice treated with X-rays + DEN, were killed at 10 weeks interval during a period of 70 weeks (for additional information, refer progress report of 1987).

Autopsies were performed and the livers were removed in toto, weighed and inspected for the presence of grossly visible discoloration and for the number and size and nodular lesions. Sections of liver, 2 to 4 mm thick, were made through the longest axis of each lobe, fixed in buffered formalin and embedded in paraffin. Three µm thick sections were cut 200 µm apart and stained with haematoxylin and eosin. Four types of focal and nodular lesions were distinguished and recorded ; foci, hyperplastic nodules, hepatocellular adenomas and hepatocellular carcinomas.

The focal lesions in 3-dimensional space were evaluated quantitatively from 2-dimensional liver intersections utilizing a MOP-VIDEOPLAN (KONTROL) image analyser. This computer was corrected to a transluscent digitizing table used either with a light cursor and a "Camera lucida" equiped Zeiss IM-35 microscope for direct measurement at microscopic resolution or as a screen for the projection of the whole section and macroscopic measurements. Calibration of the operating system was done using a calibration Zeiss Test-lines.

Mice treated with 3.1 MeV neutrons

Half of the total number of mice needed for this large scale experiment were exposed to 0.12, 0.25 and 1 Gy of 3.1 MeV neutrons. Mice were distributed in the following 4 groups :

1. Mice treated with DEN alone
   Treatment at an age of 14 days with 0 ; 0.3125 ; 0.625 ; 1.25 and 2.5 µg/g of DEN.
2. Mice treated with neutrons alone
   Treatment at an age of 7, 14 and 21 days of age with a single dose of 0.12 ; 0.25 and 0.5 Gy of 3.1 MeV neutrons.

3. Mice treated with DEN + neutrons

   Treatment at an age of 14 days with 0.3125 ; 0.625 ; 1.25 or 2.5 µg/g of DEN followed by a single dose of 0.12 ; 0.25 and 0.5 Gy of 3.1 MeV neutrons 7 days later.

4. Mice treated with neutrons + DEN

   Treatment at an age of 7 days with a single dose of 0.12 ; 0.25 and 0.5 Gy of 3.1 MeV neutrons followed by 0.3125 ; 0.625 ; 1.25 or 2.5 µg/g of DEN 7 days later.

These irradiations were performed at the new thick-target Be(d,n)-field at Ed = 6.3 MeV using a distance of ∿ 55 cm from the source and a pyramidial collimator (2.2 x 2.2 cm at the source and 14.1 x 14.1 cm at the sample position). The neutron spectrum extends to a maximum of 10.7 MeV, the average neutron energy being ∿ 3.1 MeV. The doses have been determined for each irradiation as usual with a 0.53 cm^3 thimble ionisation chamber operated in continuous TE-gas flow by total charge determination using $\langle W \rangle = (31.9 \pm 1.5)$ eV. Small temperature and pressure corrections were applied.

2. Results and discussion

Mice treated with X-rays

1. X-rays are not very effective to induce liver tumours within a period of 70 weeks, in 14 days old mice.
2. Our substrain of C57BL mice is susceptible to the induction of liver tumour by DEN within a period of 70 weeks. The induction of liver tumours is dose dependent.
3. The preliminary data seem to show that a single dose of X-rays administered to infant mouse 7 days before or after a single injection of DEN was not more effective in inducing liver tumour than DEN given alone.

Mice treated with neutrons

The experiment is still in progress. It is still too early to draw any conclusion from the first results obtained in the frame of this large scale experiment with neutrons.

IV. Objectives for the next reporting period:

1. To carry on the analysis of the final results of the large scale experiment.
2. To finalize the large scale experiment with neutrons and to carry on the analysis of the results.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

BCMN, B-2440 Geel (neutron irradiation)

Prof. Bannasch, Deutsches Krebsforschungszentrum, Heidelberg (morphological changes in the liver)

VI. Publications:

J.R. Maisin : Protection against ionizing radiation by combination of radioprotectors. Pharmacology and Therapeutics, 39 (1988) 189-193.

J.R. Maisin, S. Topalova, A. Kondi-Tamba, G. Mattelin : Radioprotection by polysaccharides. Pharmacology and Therapeutics, 39 (1988) 255-259.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-C-066-D

Institut für Strahlenhygiene
des Bundesgesundheitsamtes
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. A. Kaul
Institut für Strahlenhygiene
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Ingolstädter Landstr. 1
D - 8042 Neuherberg

Telephone number: 089-3187 5228

Title of the research contract:

Radiation-induced changes in lymphocyte populations and their functions as biological indicators of radiation damage.

List of projects:

1. Radiation-induced changes in lymphocyte populations and their functions as biological indicators of radiation damage.

Title of the project no.:

Radiation-induced changes in lymphocyte populations and their functions as biological indicators of radiation damage. Determination of membrane effects and changes of blood components after in vitro and patient exposure with ionizing radiation.

Head(s) of project:

K.W.Bögl

Scientific staff:

G.A.Schreiber, R.Hofmann, T.Butkowsky-Walkiw and A.Dehos

I. Objectives of the project:

In accidental cases of increased radiation exposure of individuals and prior to medical treatment, the actual exposure of the organism must be determined independent of physical dosimetric measurements. This necessitates a biological in vitro method (or a combination fo methods) which is specific, reproducible, significantly dose-dependent and may be quickly and simply performed in the medical laboratory without undue burdening of the patient during sampling. In addition, it should allow for dose assessment immediately after exposure as well as after a sufficiently long period of time following the exposure. The aim of this study was therefore to develop such sensitive methods for early diagnosis of radiation injury.

II. Objectives for the reporting period:

In an attempt to discover a new "biological dosemeter", several investigations have been performed to develop and test suitable indicator systems which would enable for clear and rapid dose assessment. Among others the following main features have been investigated during the reporting period:

- changes in lectin-binding of platelets, leucocytes and erythrocytes after irradiation
- changes in -amylase enzyme activities after radiation exposure of radiotherapy patients;
- radiation induced changes in the electrophoretic mobility of human erythrocytes;
- radiation induced changes in lymphocyte populations;

III. Progress achieved:

1. LECTIN BINDING SYSTEM

- INTRODUCTION

Examining the applicability of dose dependent ionizing radiation-induced effects on cells as biological indicators for purposes of dose-assessment in radiation accidents, we are specially concerned with the cell membrane. Beside electrophoretic mobility changes of human erythrocytes after X-irradiation (Butkowskyj-Walkiw et al., 1987), emphasis was put on the investigation of changes in the oligosaccharides of human blood cell membranes revealed by lectin binding. Several authors reported about dose- and time-related post-irradiation dependencies on the lectin binding capacity. However, the results are hardly comparable because of different cell types, different species and different experimental conditions (Köteles et al. 1976, Kubasowa et al. 1981a,1981b,1984, Takahashi and Kaneko 1986, Moullier et al. 1986). Also, the molecular mechanism for changes in the binding capacity is still a matter of discussion (Köteles et al. 1987). Nevertheless, the ^{3}H-concanavalin A (Con A) binding system on human blood cells (Kubasowa et al. 1981b) promised to be a skilful method for quick dosimetric statements within a therapeutically relevant dose range of 0.5 - 5 Gy - not only after in vitro but also in vivo irradiation, as tested on radiotherapy patients (Kubasowa, personal communication): Platelets, leucocytes and erythrocytes showed different degrees of radiation sensitivity, which was demonstrated by increased Con A binding within different dose ranges: 0.1 - 1 Gy, 0.5 - 2 Gy and 3.5 - 5 Gy, respectively. This allows for the possibility of dose estimation by simply comparing the binding capacity of each cell type with normal values - which are restored 24 h post irradiation - in the same individual and further means the independence from absolute values whereby normal fluctuations between individuals mostly inhibit the application of radiation-induced effects as "biological dosemeters".

This study investigated the applicability of the lectin-binding system as a "biological dosemeter". The experiments were performed on in vitro irradiated human blood cells using the same method as Kubasowa and two other radiological methods. None of these methods were suitable for confirming the described results. Changing the label from tritium to fluorescein-isothiocyanat (FITC), an approvement of this system could be achieved by measuring the binding of FITC-Con A and FITC-wheat germ agglutinin (WGA) to in vitro irradiated cells by flow cytometry. However neither different sensitivities nor applicability to radiotherapy patients could be observed. This part of the project is published by Schreiber et al.(1988).

-MATERIAL AND METHODS

Blood samples

For in vitro irradiation human blood samples were taken by venipuncture from healthy male and female donors aged 25-46 years. The blood was citrated for ^{3}H-Con A-assays or put in

12 ml EDTA-vials for flow cytometric assays.
Blood samples from radiotherapy patients were taken by venipuncture 10 min before and 30 min after irradiation and put in 12 ml EDTA-vials.

Irradiation
Blood samples in Petri dishes were placed in a water bath and irradiated with 0.5 to 5 Gy doses by X-ray machine (Isovolt Type 1501, 160 kV, 1mm Alu-filter) from a distance of 40 cm at a dose rate of 1.0 Gy/min.
Radiotherapy patients were irradiated by 8 or 15 MeV UH X-ray, or ^{60}Co -γ-rays. The integral dose (ID) was calculated according to

$$ID\ [J] = \int_{J}^{M} D\ dm$$

with D = absorbed dose at a point in the irradiated volume [Gy]
dm = mass element
M = total irradiated mass [kg]

Measured depth dose curves were used for all applied field sizes. A surface dose of 30 % of the maximum was assumed for 8 and 15 MV photons and 50 % for ^{60}Co -γ-rays. The depth of the dose maximum is 2 cm for 8 MV photons, 3 cm for 15 MV and 0.5 cm for ^{60}Co-γ-rays. For single fields the increasing part of the depth dose curve was approximated by a straight line between 30 %- or 50 %- and 100 % values. The decreasing part of the curve was also approximated by a straight line determined by the maximum dose and the dose in the reference point. Mean values of the dose in the ascending and descending parts of the depth dose curve were determined and utilized for the calculation of the ID in the irradiated volume. For opposing fields the ID was calculated for the volume lying within the dose plateau as a first approximation. The contributions of the build-up regions result from the fact that the average absorbed dose turned out to be 80 % of the reference dose there. For the calculations of the irradiated masses from the corresponding volumes unit density was assumed (1 g/ml), neglecting any influence of bones. The divergence of the beam was also neclected. Shielded volumes were considered to exhibit a transmission of 10 % of the reference dose.

Fixation and separation of blood cells
30 min after in vitro or in vivo irradiation, 1 vol 0.05% glutaraldehyde in PBS was added to the blood samples as well as to the controls for a mild fixation of cell surfaces for 20 min. After fixation, 1 vol PBS, pH 7.2, was added and 4 ml of the cell suspension was layered on top of a 3 ml Ficoll-gradient (Pharmacia) to be centrifuged 25 min at 420 g. The leucocyte-fraction which contained also the platelets and the erythrocyte-fraction were taken, PBS, pH 7.2, added and centrifuged 10 min at 130 g. The platelets in the supernatant

of the leucocyte-fraction were pelleted 10 min at 750 g and washed twice in PBS. Leucocytes and erythrocytes were also washed twice in PBS and pelleted 10 min at 80 g. All centrifugations were carried out by placing the 15 ml glass vials in a swing-out rotor.

Binding of ^{3}H-Con A to the blood cells

10^6 cells in 100 µl PBS, pH 6.8, were incubated at 20°C with 100 µl PBS, pH 6.8, (1 µCi/ml) ^{3}H(G)Con A (20 Ci/mmol, NEN) or (2 µCi/ml) N-[acetyl-^{3}H]acetylated Con A (43.5 Ci/mmol, Amersham). To determine the specific binding, ^{3}H-Con A was preincubated with 0.1 M methyl-α-D-mannopyranoside (Sigma) for 1 h. All results are expressed as the mean value of three parallel samples with standard deviations (SD).

Separation of cell bound and free ^{3}H-Con A

1. Washing in PBS

After 10 min incubation of cells and ^{3}H-Con A in BSA-saturated 15 ml glass vials, 10 ml PBS, pH 6.8, were added. Leucocytes and erythrocytes were pelleted 5 min at 750 g, platelets 5 min at 1750 g. All cell fractions were washed twice. The resuspended cells in 0.5 ml PBS were incubated with 0.5 ml 0.5 M NaOH for 2 h. Radioactivity was measured after transfer in 15 ml scintillation mix (Quickszint 2000, Zinser Analytik, Scintillationcounter LS 7800, Beckmann) in cpm.

2. BSA-separation-technique

2.3 ml 5% BSA-solution (w/v PBS, pH 6.8, Sigma) were put in 2.2 ml microtubes (Eppendorf) for at least 1 h to saturate unspecific binding sites. After centrifuging the solution at 8000 g, to inhibit binding of pelletable particles to ^{3}H-Con A, 300 µl BSA-solution were removed and the two components of the binding assay were layered on top of the BSA-cushion. After 20-24 min, i.e. after saturation of binding, the cells were slowly centrifuged through the separation medium to inhibit turbulencies. Leucocytes and erythrocytes were centrifuged 5 min at 900 g, platelets 5 min at 1700 g in a swing-out rotor. The solution was sucked off and the pellet was resuspended in 150 µl 0.5 M NaOH and incubated for 2 h, before 2 ml scintillation mix was added to the microtube. The microtubes were placed in 20 ml empty scintillation vials for measuring the radioactivity in cpm.

To investigate the distribution of radioactivity throughout the BSA-solution in dependence of centrifugal power, the solution was taken in fractions.

3. Cell Harvester-technique

The binding assay was carried out in BSA-saturated 96-well microtiter plates (Falcon) and transfered with a 12-channel cell harvester (Skatron) after 10 min on a BSA-saturated glass fiber filter (GF/B, Whatmann). The filters were put in 6 ml scintillation vials and incubated with 200 µl 0.5 M NaOH for 2 h. After adding 4 ml scintillation mix the vials were placed in 20 ml empty scintillation vials for measuring the radioactivity in cpm.

FITC-Con A- and FITC-WGA-binding to cells

10^6 cells in 100 µl PBS, pH 6.8, were incubated in 15 ml glass vials with 100 µl PBS, pH 6.8, containing 500 nM FITC-Con A or 250 nM FITC-WGA (Sigma), for 10 min at room temperature. For three times washing 10 ml PBS were added and leucocytes and erythrocytes were pelleted 5 min at 750 g, platelets 5 min at 1750 g. To determine the specific binding, FITC-Con A was preincubated with 0.1 M methyl-α-D-mannopyranoside (Sigma), FITC-WGA with N-acetyl-D-glucosamine (Sigma), for 1 h. The samples were held in dark and on ice till measurements. 10^4 cells were recorded on a FACS-Analyser (Filterset FITC/PE, Becton Dickinson) with a Consort 30 program (Hewlett-Packart). For adjustment, 1 µm and 5 µm fluoresbrite carboxylated microspheres (Paesel) were used. To analyse cell type specific FITC-fluorescence (FL1), the cells were gated in the VOL/SSC (light scatter)-Plot. The lymphocytes and monocytes were separately analysed in the leucocyte-fraction. The FL1-histograms of irradiate samples were compared with the controls by the Kolmogorov-Smirnov-statistic (Young 1977), which expresses the differences between two histograms as D/s(n)-value. These values were defined as positive (binding capacity increase), when the sample integral was right of the control integral and vice versa. The experimental deviations were determined cell type- and lectin-specific by 6-time recording of same samples and parallel samples and noted in the diagrams as marked area.

-RESULTS

With the same method as that used by Kubasowa, the binding capacities of human platelets, leucocytes and erythrocytes after in vitro X-irradiation were measured in the dose range of 0.5 - 5 Gy. The major problem of this method was the large standard deviations making it nearly impossible to see any correlation with the published results. It seemed that the results were neither reproducible between donors nor on cells of the same donors measured on different days. The data are listed in Schreiber et al. (1988). The large standard deviations were obviously caused by the experimental procedure, especially by loss of cell material during the intensive washings in phosphate-buffered saline (PBS) to separate the free from the bound radioactivity. Moreover, the quantitative recovering of the 106 cells after pelleting in 15 ml glass vials for transfer to the scintillation cocktail is susceptible to inaccuracies.

Thus, and to gain an opinion on this "dosemeter", it was tried to reduce the deviations by two other methods using ^{3}H-Con A. The first is based on the "microfuge method" of Phillips and Furmanski (1976). After our modification of this method, it is possible to do the binding assay on the top of a bovine-serum-albumin (BSA)-cushion and separate the free from the bound radioactivity quantitatively in one centrifugation step. Furthermore, by saturation of unspecific binding sites of the tube with separation medium BSA, it is possible to quantitatively remove the free ^{3}H-Con A and thus perform binding assay, separation and scintillation counting in the same vial, which minimizes the loss of cell material.

Performing the binding assay in microtiter plates for transfer on BSA-saturated

glassfiber-filters by a cell harvester, achieved the smallest standard deviations. As with the BSA-centrifugation-technique, the control experiments showed a high degree of reproducibility (Schreiber et al. 1988), but the dose-dependency of the cell types' binding capacities was not sufficient to base a dose assessment on these results. Appraising those three methods, it should be stated that measurable radiation-induced changes of oligosaccharides in the dose range we are looking for are too small for reproducibility in whole cell populations. It is assumed that by adding the binding capacities of cells, as with these radiological methods, the effect measurable on possibly only a small number of cells may be lost in the pool of undamaged binding sites.

By performing single cell binding capacity measurements in a flow cytometer, using the FITC-labeled lectins Con A and WGA, increased binding capacities could be observed on platelets, lymphocytes and monocytes for both lectins already after in vitro irradiation with a dose as low as 0.5 Gy. The analysis of the fluorescence-histograms was done by Kolmogorov-Smirnov-statistics, expressing the difference between histograms as D/s(n)-value. This increase is reproducible not only on one donor measured on different days but also on various donors (Fig. 1). The fluorescence intensities of all three cell types reached a plateau between 1 and 5 Gy, which was significantly higher than the unirradiated control level. The changes of erythrocytes are within the control range.

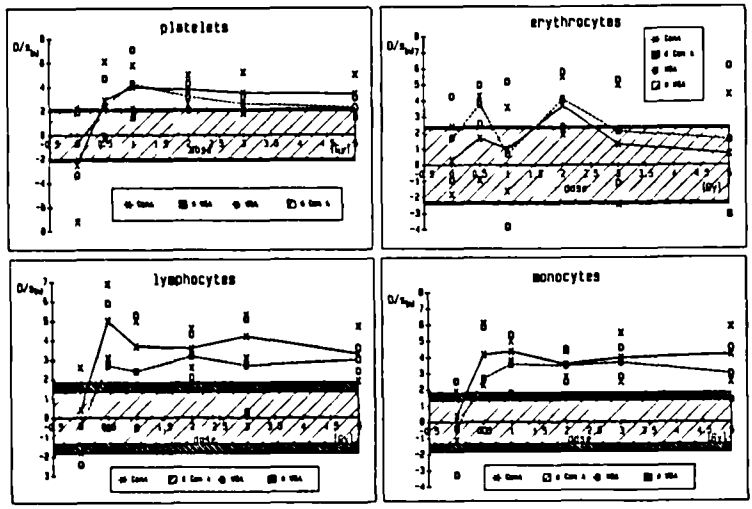

Figure 1. Determination of the binding capacities of platelets, lymphocytes, monocytes and erythrocytes of six male and female donors to FITC-Con A and FITC-WGA after in vitro irradiation of the blood samples. The SD and the experimental deviations (d Con A/d WGA) are indicated as points and marked area, respectively.

Thus, although there was clearly an increase in binding capacities also with this technique, the different radiosensitivities of the blood cell types could not be confirmed. The increased binding of platelets, lymphocytes and monocytes however revealed an irradiation in at least

the dose range of 1 - 5 Gy.

To transfer in vitro data to in vivo conditions, blood samples were taken from radiotherapy patients before and after the first irradiation and investigated as to their suitability for dosimetric statements. But even though all patients had been irradiated in the actual dose range between 1 - 5 Gy, there is no notable dose-related dependency on any of the different blood cell types of partly-body irradiated patients. Furthermore, there are also no significant changes between the cells of partly-body and whole-body irradiated patients whose integrale doses were about 10 to 100 times higher (Fig. 2).

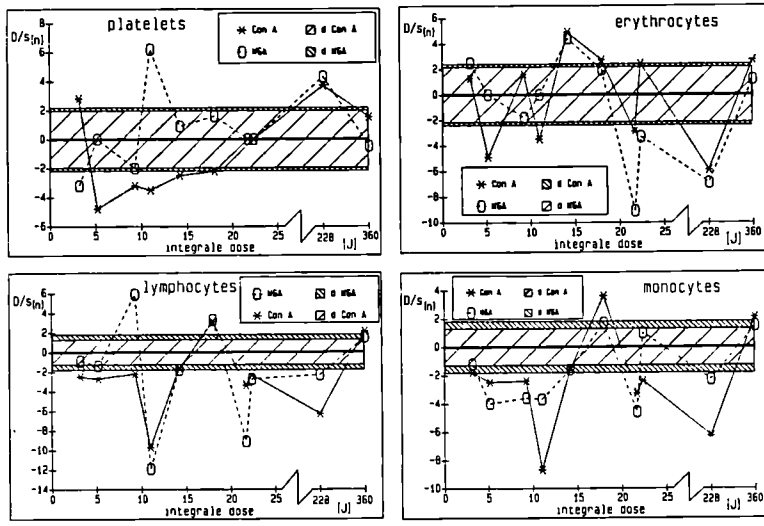

Figure 2. Binding capacity changes of platelets, lymphocytes, monocytes and erythrocytes of partly body irradiated (3.2 - 22.4 J) and whole body irradiated (228 - 360 J) patients. The experimental deviations (dConA/dWGA) are indicated as marked area.

-DISCUSSION

Biochemical indicators should reveal accidental irradiations of human beings after a few hours, but their applicability is mostly limited or inhibited due to the fluctuating values of the investigated parameters between individuals (Butkowskyj-Walkim et al. 1987, Hofmann et al. 1987,1988). The described lectin-binding system on in vitro irradiated human blood cells (Kubasowa et al. 1981b) promised to be - apart from its short analysing time - a "biochemical dosemeter" independent from normal individual fluctuations, because the dose assessment was done on the basis of different sensitivities of the blood cell types rather than on the absolute changes of the binding capacities. However, not only were dose assessments made on in vitro irradiated cells but also on in vivo irradiated cells taken from radiotherapy patients.

Our measurements performed with the method used by Kubasowa could not confirm these results. Furthermore, it was nearly impossible to make any statements because the values were burdened with large standard deviations. But also the other two methods based on

^{3}H-Con A did not yield reproducible results - although the standard deviation was lowered to about 10 %. The reason we assumed was that the measurable changes in the oligosaccharides were too small to be seen on a whole cell population.
Thus, if this lectin-binding system owns "biologial dosemeter" potency, then it should be revealed by single cell measurements of the binding capacity.
This was done on a flow cytometer able to differentiate 256 fluorescence-intensities, followed by an analysis of the histograms by Kolmogorov-Smirnov-statistics (Young, 1977). This nonparametric analysis compares the integrales of two histograms which means that changes over the whole course of the curves are ascertainable. The D-value expresses the vertical displacement of one integral to the other. To compare histograms composed from different cell numbers the D/s(n)-value must be calculated. The D/s(n)-values, which counts for significant irradiation induced differences, were emperically estimated by repeated recording of same and parallel samples.
On this basis it could be shown that the binding capacities of in vitro irradiated platelets, lymphocytes and monocytes for FITC-Con A and FITC-WGA were significantly increased in the dose range between 1 - 5 Gy. But again no different sensitivities could be seen. This means that the incident of irradiation can be proved through the binding behaviour of these three cell types. This result did not fulfill the hope for a more precise dose assessment. Nevertheless, the medical need for dosemeters demanded an in vivo investigation of the potency of this system. These investigations can normally only be done on radiotherapy patients. However, one should consider that the investigated parameters of these patients may already have been influenced by the disease or by earlier therapies. But, also if these investigations may serve only as a model for the normal situation, tendencies should at least be indicated.
On partly-body irradiated patients, no dependence of binding capacities on applied doses could be observed. This may be due to the low integral dose. But the absence of a significant difference between the binding capacities of cells of partly- and whole-body irradiated patients leads us to believe that the Con A- and WGA-binding system will not fulfill the requirements needed for a dosimetric system in the investigated dose range.

2. SERUM AMYLASE SYSTEM

The increase of sugar hydrolysing enzyme amylase (salivary type) concentrations in blood appears to be a suitable indicator of irradiation shortly after exposure. Our results and the results of other working groups indicate a significant (P 0.1) increase from 1 Gy onward. Provided an irradiated gland volume of 50 % exists, it seems to be possible - as indicated in table 1 - to recognise the radiation event. This is true for all amylase tests quoted in table 1.

Table 1. Statistical evaluation of serum amylase activities before and after irradiation (18 - 30 hours) of 164 radiotherapy patients.

number of persons	with serum amylase activity a) totally	b) pre irr. level > normal range	c) post irr. levels > normal range	d) pre irr. levels > 3 x mean value of normal range	e) post irr. levels > 3x mean value of normal range
literature:					
Hofmann et al.	41	7*	38	3	38
Willich et al.	+ 50	3	46	1	34
Junglee et al.	6	-	6	-	6
Barret et al.	12	1	12	1	12
Chen et al.	16	2	13	-	11
Kashima et al.	23	1	22	-	21
Van den Brenk et al.	16	-	16	-	16
totally	164	14	153	5	138
(%)	(100)	(8.5)	(93.3)	(3)	(84.2)

* high amylase activities are probably caused by drug treatment (dexamethasone). After irradiation all of these patients show an at least 3-fold increase of the amylase value compared to the mean of the normal range, and in 6 cases an even more than 3 fold increase of the starting point activity (prior to irradiation).

+ only patients with irradiation doses 1 Gy

Table 1 shows an evaluation of serum amylase data 18-30 hours post irradiation compared to the normal range of the specific amylase assay. 93.3 % of the irradiated cohorte had greater amylase levels than the normal range. However, 8.5 % of these patients showed elevated amylase levels already before irradiation. This means, in case of application as biochemical irradiation indicator, there would be a wrong positive judgement in 8.5 %. The results refer to several different working groups which investigated the radiation-induced increase of the enzyme activity. However, a direct irradiation of the salivary glands is essential. This means, for practical application as biochemical radiation indicator, that it is impossible to differentiate between whole body- and partial body irradiations. This is also shown in figure 3: 24 patients irradiated in the head and neck region (4 Gy tumour dose) are compared with 17 whole body irradiated (4 Gy whole body dose) persons. The distribution of the 24 hours amylase blood levels as well as the amount of amylase increase were comparable. But the strong variations, even at comparable irradiation doses, in the amount of blood amylase

increase indicate that the serum amylase system can serve only as a bioindicator and not as a "dosemeter".
This part of the project is published by Hofmann et al.(1987).

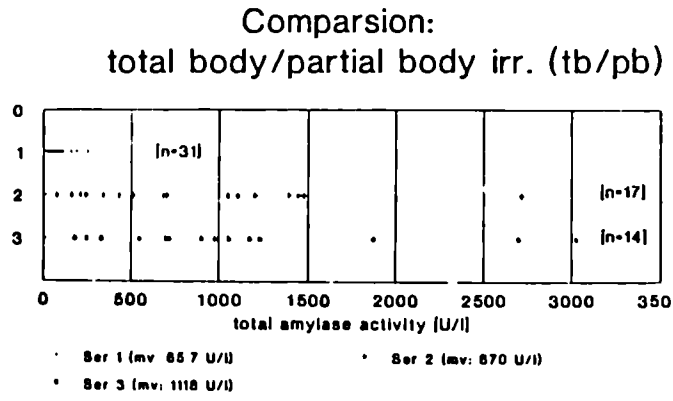

Figure 3. Serum amylase activity distribution before and after irradiation

3. RADIATION INDUCED CHANGES IN ELECTROPHORETIC MOBILITY OF HUMAN ERYTHROCYTES

Human erythrocytes were utilized to investigate radiation induced changes in the electrophoretic behaviour with the help of free-flow electrophoresis. Analysis was based on the radiation induced change in the electric charge of the erythrocyte membrane. Due to the presence of sialic acids, the cell membrane has a negative charge. If cells are exposed to ionizing radiation, the resulting change in their surface charge will lead to a change in electrophoretic mobility (EPM). Sato (1977) suggested a temporary postirradiation disorder of the membrane structure due to the dislocation of sialic acid from the peripheral zone of 0 - 7.5 Å to a deeper zone of 9.17 -17 Å. Also we found that the character of the radiation induced alterations is transient. Thus, it seemed to be of practical benefit to devise a method of fixation of the radiation-affected cell surface.
In vitro irradiated (0.8 to 5.0 Gy) red blood cells show a change in electrophoretic mobility (EPM) after fixation with $KMnO_4$ in a low dose range of 3 to 5 Gy during the first 2 days after exposure.
In vivo experiments, performed on radiotherapy patients, indicate an increase up to 6 % in EPM after a whole body irradiation with 4 Gy. Also this effect can be observed up to 2 days after radiation exposure. But due to the variation in EPM of non-irradiated erythrocytes this

effect is not large enough to base a dose-assessment on it. This part of the project is published by Butkowskyj-Walkiw et al.(1987).

4. MEASUREMENT OF THE LYMPHOCYTE SUBPOPULATIONS

In this project research was first conducted into one of the immunological parameters - i.e. the lymphocyte subpopulations.

Since a flow cytometer (FACS Analyzer, Becton Dickinson) is now available at our Institute, the method applied up to now for lymphocyte differentiation (i.e. rosette formation using antibody-labelled latex beads), was replaced by the faster and more accurate method based on immunofluorescence and flow cytometry. However, we only used non-irradiated mononuclear cells from healthy donors to test the apparatus.

A comparison of the rosette technique and flow cytometry, based on the results obtained for T-cells, T-helper/inducer cells and T-suppressor/cytotoxic cells in samples from healthy donors is given in table 2. However, this is not a direct comparison, since the data are not based on identical groups of individuals, and it is, therefore, only to a limited extent possible to evaluate the manual method in comparison to the automated count, which is more accurate (due to the greater number of cells).

Nevertheless, it may be concluded that the values for T- and B-cells show good correlation, while the T-helper/inducer cells and the T-suppressor/cytotoxic cells show more important variations which might be due to the more difficult procedure of manual counting.

Table 2. Comparison of the results obtained for T-cells, B-cells, T-helper/inducer cells and T-suppressor/cytotoxic cells in healthy individuals using the rosette technique vs. flow cytometry.

	rosette technique			flow cytometry			
subpo-pulation	% of lymphoc. mean	range	number of donors		% of lymphoc. mean	range	number of donors
T-cells	74.8	62-82	26	Leu4	73.5	63-83	6
				Leu1	71.3	67-77	4
B-cells	12.4	8-22	26	Leu12	11.0	5-15	5
T-helper/inducer	50.6	39-61	8	Leu3	45.8	39-59	14
T-supp./cytotox.	19.3	14-23	8	Leu2	23.8	12-41	14

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V. Publications:

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G.A. Schreiber, A. Spiegelberg, K.W. Bögl. Die Lektinkopplung als "biologischess Dosimeter"?

3. Symposium über molekulare und zelluläre Mechanismen der biologischen Strahlenwirkung, Neuherberg/München, 23.-25.4.1988, Kurzreferateband, p 66.

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RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

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Contract no.: BI6-C-069-B

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Title of the research contract:

Morphological and cytogenetical studies on the sensitivity of the mammalian embryo to low doses of radiation.

List of projects:

1. Morphological and cytogenetical studies on the sensitivity of the mammalian embryo to low doses of radiation.

Title of the project no.:

Morphological and cytogenetic studies on the sensitivity of the mammalian embryo to low doses of radiation

Head(s) of project:

P. Jacquet

Scientific staff:

P. Jacquet, S. Grinfeld, L. Baugnet-Mahieu, A. Léonard

I. Objectives of the project:

In vitro culture of embryos represents a simple model system to analyse very precisely the modifications of radiosensitivity occuring during the preimplantation period. The one-cell embryo has been little studied, and results obtained so far showed that this stage is particularly radiosensitive. However the techniques utilized for these investigations were very different and implied, i.e. in vitro or in vivo fertilization and irradiation, use of natural or hormone- stimulated ovulation. In addition, hybrid or inbred strains of varying radiosensitivities were used. Such variations in the experimental conditions could explain, at least partially, important discrepancies reported between laboratories. In this project, we intended to study the influence of these factors, and the mechanisms by which they can modify the response of the embryo to X-irradiation.

Ii. Objectives for the reporting period:

Our last results on irradiated embryos suggested that caffeine could reverse G2-arrest by stimulating the synthesis of some protein(s) necessary for division. We intended to verify this hypothesis, using highly synchronized embryonic populations, and incubating them with cycloheximide and caffeine according to different schemes, but also by comparing protein synthesis and phosphorylation in control and irradiated embryos incubated or not with caffeine. We also intended to determine if there exists a threshold dose for the occurence of G2-arrest. Thus, the occurence of a G2-dealy, as well as its length, would be studied after irradiation with very low doses of X-rays. Cytogenetic investigations would be performed on embryos given the lowest doses inducing G2-arrest. These studies have just started and will not be commented.

III. Progress achieved:

1. Methodology

Animals, irradiation

The necessity to dispose of highly synchronous embryonic populations appeared from our last studies. Thus, females were usually superovulated and mated only during a restricted period of two hours in the morning, after all oocytes had been ovulated. Fertilization was considered to occur one hour after the beginning of the mating period and irradiation of the pregnant females with various doses of X-rays was performed 8 hours later.

Analysis of polypeptides synthesis and comparison with morphological studies

Polypeptide synthesis and phosphorylation were studied in normal or blocked embryos (2.5 Gy) by two-dimensional electrophoresis. Embryos were collected immediately after irradiation, and only those showing a second polar body were conserved. Those were cultured in normal medium until at least 3 hours before the time of normal division, at which various types of incubations were initiated (incubations with or without caffeine and cycloheximide, in the presence of ^{35}S or ^{32}P). Incubation with caffeine and/or cycloheximide lasted generally 3 or 6 hours, while that with ^{35}S and ^{32}P was limited to 3 hours. Other embryos were used for morphological studies. Incubations with or without caffeine and cycloheximide were initiated at the same times as for biochemical studies, but they were continued until at least 2 days after fertilization. Embryos were regularly examined to analyse precisely the effects of the different treatments on the first cleavage and G2-arrest.

Studies on the G2-arrest after very low doses of X-rays

Pregnant females were irradiated with decreasing doses of X-rays, their embryos were collected and examined at 2 hour-intervals, from the time of presumed first cleavage. At each control, cleaved embryos were separated from the other ones, and their development was followed to the blastocyst stage.

2. Results

Analysis of polypeptide synthesis and comparison with morphological studies

The results and conclusions which may be drawn from these experiments can be summarized as follows :

(1) Irradiated embryos suffer a certain delay in protein synthesis : at 18h30 after fertilization, control embryos have completed all protein synthesis necessary for cell division, while it is not true for irradiated embryos.

(2) We assume that the inability of irradiated zygotes to enter the mitosis is linked to an absence of specific phosphorylation of some polypeptides of 35 KD molecular weight, whose synthesis has begun earlier in the cell cycle.

(3) We propose that, normally, the phosphorylation of these polypeptides is under the dependence of a control mechanism, which initiates the process when the quantity of the polypeptides has reached a critical

level : this level is never reached when irradiated embryos are incubated from 18h30 after fertilization in medium containing cycloheximide, so that almost no embryo will divide, even after the normal delay, unless caffeine is added to the medium.
(4) When blocked embryos are incubated from 18h30 in the presence of caffeine, the phosphorylation of the 35 KD polypeptides clearly appears on the autoradiographs, and their appearance immediately preceeds the division.
(5) Moreover, caffeine is able to induce division of blocked embryos even in the presence of cycloheximide. In these conditions, phosphorylation of the 35 KD polypeptides is still seen, although at a lesser degree, since it only concerns those polypeptides which had been synthesized earlier during the cell cycle. In addition, the proportion of embryos stimulated to cleave is lower than when cycloheximide is absent from the medium, and embryos which are not cleaved after the period of normal cleavage will not anymore cleave after G2 delay, if cycloheximide is maintained in the culture medium together with caffeine.
(6) We suggest that caffeine could act, directly or indirectly, by bypassing the control mechanism of phosphorylation of the mitotic polypeptides, forcing the phosphorylation whatever the level reached by the polypeptides. The division would occur only at a certain level of phosphorylation. In other words, cell division would occur only if the quantity of polypeptides which are disponible for phosphorylation is sufficient. Under certain experimental conditions, this requirement is satisfied in some embryos, but not yet in others. Thus, in the presence of caffeine, the requirement of blocked embryos for protein synthesis is alleviated, although not completely suppressed.

Studies on the G2-arrest after very low doses of X-rays

The results of our experiments showed that doses as low as 10 cGy can still induce a detectable G2-arrest in embryos. In contrast with the results obtained after doses of 1 Gy or more, where the delay was always equivalent to one entire cell cycle, large individual differences were found after application of low doses of radiation. Interestingly, embryos suffering even a slight delay were unable to survive up to the blastocyst stage, whatever the dose administered. Doses of 5 cGy did apparently not anymore induce a G2-delay.

3. Discussion

The radiation-induced G2-arrest is usually considered as a passive consequence of a damage to a proteinaceous "target", whose integrity would be a prerequisite for progression to mitosis. Our results show that, in some conditions, the restitution of such a "division"-protein, or more broadly, repair of the lesion(s) responsible for G2-arrest is not an absolute requirement for G2 cell progression. Thus, caffeine could, directly or indirectly, render functional some polypeptides which could play an important role in the sequence of events leading to the first cleavage, by forcing their phosphorylation. Investigations realized with very low doses suggest that the treshold dose for the G2-delay would be localized around 5-10 cGy, and that the zygotes suffering even a slight division delay are unable to survive to the blastocyst stage.

IV. Objectives for the next reporting period:

Cytogenetic studies in delayed embryos given very low doses of X-rays will be continued, to determine (1) if they also show abnormally high levels of chromosome anomalies and (2) if the level of chromosome anomalies is modified during G2-delay. Zygotes can normally divide even in the absence of a nucleus. We intend to use this property to determine conclusively if the delay is a passive consequence of radiation, resulting from damage to the processes necessary for progression, or an active response triggered by cellular defects not directly concerned with progression, but presumably advantageous for the cell to repair before mitosis. Actually, and in contrast with some other investigators, we think that G2-delay is a passive consequence of irradiation. Absence of delay after irradiation of enucleated zygotes would, however, signify that the target is located in the nucleus, and that G2-arrest is an active response to irradiation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

1. Publications in scientific journals

S. Grinfeld and P. Jacquet : G2-arrest in mouse zygotes after X-irradiation : reversion by caffeine and influence of chromosome anomalies. Int. J. Radiat. Biol. 54, 257-268 (1988).

S. Grinfeld, P. Jacquet, J. Gilles and L. Baugnet-Mahieu : The X-ray induced G2-arrest in mouse eggs : a maternal effect involving a lack of polypeptide phosphorylation. Roux's Arch. Dev. Biol. 197, 302-304 (1988).

2. Communications

P. Jacquet, S. Grinfeld, L. Baugnet-Mahieu and J. Gilles : Studies on the mechanisms of X-ray induced G2-arrest in the mouse zygote. 16th Conference of European Teratology Society, Baveno (Italy), September 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

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Title of the research contract:

Contribution of flow cytofluorimetry for the assessment of over-exposure to ionizing radiation.

List of projects:

Contribution of flow cytofluorimetry for the assessment of over-exposure to ionizing radiation.

Title of the project no.: BI 6 - 073 - F

CONTRIBUTION OF FLOW CYTOMETRY FOR THE ASSESSMENT OF OVER-EXPOSURE TO IONIZING RADIATION.

Head(s) of project:

Dr. R. MASSE

Scientific staff:

G. FRELAT
M. N. GUILLY
J. PRUDHOMME

I. Objectives of the project:

The evaluation of ionizing radiation overexposure has been made, until now, by measuring chromosomal aberrations in the mitotic lymphocytes of irradiated individuals. More recently, a method taking into account the radio-induced membrane modifications of different subpopulations of blood cells has been described.

Our aim is to adapt these valuable methods, but heavy and tedious, for flow cytometry analysis with the expected following benefits: rapidity, analysis of large number of biological objects, multiparametric analysis, immediate statistical analysis, correlation between different parameters, etc...

The purpose of the project is to evaluate the potentiality of flow cytometry towards 3 main goals: heterogenous chromosomal aberration detection on flow karyotypes, use of anticentromeric antibodies for detection of a- and dicentric chromosomes, and measurement of membrane modifications by fluorescent probes (lectins, monoclonal antibodies, etc...).

II. Objectives for the reporting period:

The objectives for the reporting period concentrated mainly on the second goal of the project: centromeric detection with fluorescent antibodies. It was shown in the preceeding reporting period, that some chromosomes may have higher levels of FITC fluorescence. It was thus hypothesized that this phenomenon could be due either to contaminant antibodies recognizing antigens other than kinetochorial proteins or to kinetochores with different sizes varying with the chromosomal length. It was thought that the availability of monoclonal antibodies may allow to discreminate between these hypotheses and facilitate the flow cytometric detection of dicentric chromosomes.The aim was to obtain a biological reagent better than crude human antiserum or purified human IgG to solve the problem of important FITC background when labelling chromosomes in suspension.

III. Progress achieved:

In order to achieve a reduction of the unspecific fluorescence background during chromosomal centromere labelling in suspension, several approaches were investigated.

A - MOUSE MONOCLONAL ANTIBODIES

1 Methodology

Thanks to the courtesy of Dr. Earnshaw (Johns Hopkins University, Baltimore, Maryland, USA), 2 mouse monoclonal antibodies anti-CENP-B (one of the proteins of the kinetochore) were used for the centromere labelling of chromosomal suspensions from the two human lymphoblastoid cell lines ICB 100 and 101 previously employed (see "Euratom Progress Report 1987", p. 705-710). These antibodies recognized different epitopes of a fusion protein obtained through the expression of a cDNA containing part of the gene CENP-B and part of the bacterial .-galactosidase gene (Earnshaw W.C. et al., J Cell Biol, 1987, 104: 817-829). The second fluorescent FITC-labelled antibodies were sheep anti-mouse IgG. Indirect immunofluorescence was also performed on HeLa cells grown on coverslips as described for the characterization of new nucleus specific autoantibodies (Guilly M.N. et al., Eur J Cell Biol, 1987, 43: 266-272).

2 Results

None of these mouse monoclonal antibodies gave centromeric labelling on chromosomes in suspension with the method already used with human sera from patients with the CREST syndrome of scleroderma.

Various dilutions of these mouse monoclonal antibodies were tested on HeLa cells by indirect immunofluorescence microscopic analysis. A positive staining of kinetochores was only observed at low dilutions (1:10) contrary to human antiserum which still gave positive results at high dilution (1:10 000). For the sake of comparison the same human serum was used for chromosomal labelling in suspension at dilutions between 1:250 and 1:500.

3 Discussion

The negative results obtained on chromosomal suspension with these mouse monoclonal antibodies anti-CENP-B are understandable when comparing the difference in affinity observed on HeLa cells between them and human serum. The apparent low affinity of these mouse monoclonal antibodies may originate from trivial reasons such as lyophylisation/rehydratation difficulties. But as for the same human sera affinity differencies were observed between in situ and in suspension labelling techniques, it is very likely that the epitopes recognized on the fusion protein or on the cells grown on coverslips are more or less either hidden or modified when the chromosomes are released from the cells into the flow analysis buffer.

These results raise questions about the best biological reagents suitable for specific, high affinity, labelling of chromosome centromeres in suspension. It was thus decided, before searching for human monoclonal antibodies, to come back to human anti-kinetochore sera with 3 objectives: improvement of chromosomal suspension labelling method with new human antisera; characterization of human kinetochore proteins by immunoblotting and immunoprecipitation; purification of these proteins in order to produce new monoclonal antibodies and/or polyclonal sera.

B - HUMAN SERA

1 Methodology

Through collaboration with Dr F. Danon (Laboratoire d'Immunochimie et d'Immunopathologie, Hopital St Louis, Paris), 59 human sera from patients with the CREST syndrome of scleroderma were available. Indirect immunofluorescence was performed, as described in the preceeding section, on HeLa cells grown on coverslips, except that the second fluorescent FITC-labelled antibodies were sheep anti-human IgG. For biochemical characterization of the sera, immunoblotting and immunoprecipitation of nuclear proteins from KE37 cells were performed as reported for lamins (Guilly et al., 1987, see above).

2 Results

All of the 59 sera were tested at various dilutions on HeLa cells. Though all were characterized as antikinetochore-specific by immunofluorescence analysis, the FITC background differed from one serum to another. Biochemical characterization of 17 sera was undertaken by performing immunoblotting and immunoprecipitation of the recognized nuclear antigens from a human T lymphoblastoid cell line (KE37). On immunoblots, most of the sera (11/17) detected the 80 kD nuclear protein (CENP-B). The antibodies eluted from this nitrocellulose-immobilized antigen labelled the kinetochores on cultured cells. However an important background level and a strong non-specific staining of the histones were often observed with this technique. Immunoprecipitation of biosynthetically labelled nuclear proteins was carried out to overcome the problem of non-specific binding. This method allowed the identification of 8 other proteins having the following molecular weights: 21, 51, 65, 70, 75, 110, 150 and 200 kD, which were also recognized by some of the 17 CREST patients' sera.

3 Discussion

The variation of FITC background between sera reinforces the need to select the best one and to determine accurately the optimal concentrations for autoimmune sera and FITC-labelled second antibodies. The results from immunoblotting and immunoprecipitation experiments show that antigens other than the 80 kD protein may be recognized by the patients' sera. As the mercury arc lamp used in fluorescence microscopy has a poor exciting capacity for FITC, the human eye cannot distinguish very low fluorecence level which can be revealed by laser illumination. For example, the detection limit of the flow cytometer used (ATC 3000) is 700 true FITC molecules (Gaucher et al.,1988, this study). Therefore it is important to characterize biochemically the antigens recognized by the CREST sera before looking for monoclonal or polyclonal antibodies. In this respect the immunoprecipitation of biosynthetically labelled nuclear proteins seems to be a good technique. The obtention of pure antigens from chromosomal centromeric regions may solve the necessity of a choice between monoclonal antibodies (specific, low affinity) and polyclonal antibodies (less specific, high affinity). Another approach could be the use of a slit-scan flow cytometer which allows the recognition of the fluorescence profile of each chromosome. The possibilty of setting a fluorescence intensity threshold should permit the unambiguous detection of dicentric chromosomes.

IV. Objectives for the next reporting period:

Work in the next reporting period will still concentrate mainly on the labelling of chromosomal kinetochores. For that purpose, researches will focus on the confirmation of the preliminary biochemical results, on the analysis of the antigenic specificity of other autoimmune sera, on the purification of immunoprecipitated antigens for the production of murine monoclonal antibodies and polyclonal sera and on the use of immunoadsorbed sera. On the other hand analysis of chromosomes with labelled kinetochores will be performed on the Lawrence Livermore National Laboratory (USA) slit-scan apparatus in collaboration with Dr. B. Trask.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs F Danon and JC Brouet: Laboratoire d'Immunochimie et d'Immunopathologie, INSERM U 108, Hopital St Louis, Paris.
Dr JC Courvalin: Centre de Génétique Moléculaire. CNRS. Gif sur Yvette.
Drs JC Gaucher and A. Seigneur: Département d'Electronique Industrielle et Nucléaire. Service d'Instrumentation pour la Recherche. CEN Saclay. BP4. F91191 Gif sur Yvette Cedex.
Dr MT Doloy: DPS. SPE. Laboratoire de Physiopathologie Expérimentale. CEN FAR. BP6. 92265 Fontenay aux Roses Cedex.

VI. Publications:

* GAUCHER J.C., GRUNWALD D., FRELAT G. (1988)
"Fluorescence response in flow cytometry: sensitivity determination for ATC 3000 cytometer"
Cytometry, 9 , 557-565.

* GRUNWALD D. (1988)
"La cytogénétique en flux chez les grands mammifères: Application chez le porc et le singe Cynomolgus"
in: "La cytométrie en flux, pour l'étude de la cellule normale ou pathologique." Ph. Metezeau, X. Ronot, G. Le Noan-Merdrignac, M.H. Ratinaud (eds.). Editions MEDSI/Mc GRAW-HILL. Paris. Vol.1. Chap. 25. p. 277-284.

* DELATTRE O., GRUNWALD M., BERNARD A., GRUNWALD D., THOMAS G., FRELAT G., AURIAS A. (1988)
"Recurrent t(11;22) breakpoint mapping by chromosome flow sorting and spot blot hybridization"
Human Genetics, 78 , 140-143.

* SCHMITZ A., OLSCHWANG S., CHAPUT B., THOMAS G., FRELAT G. (1988)
"Oncogene detection by enzymatic amplification on flow sorted chromosomes"
Nucleic Acid Research, 17 (2). (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-074-UK

United Kingdom Atomic Energy
Authority
11 Charles II Street
GB - London SW1Y 4QP

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Morgan
Env. & Med. Sciences Division
Harwell Laboratory
Didcot
GB - Oxon OX11 ORA

Telephone number: 0235-24141 (Ext. 4622)

Title of the research contract:

Macrophage involvement in actinide-induced lung disease.

List of projects:

1. Macrophage involvement in actinide-induced lung disease.

Title of the project no.:

Macrophage involvement in actinide-induced lung disease.

Head(s) of project:

A Morgan

Scientific staff:

N D Priest
R J Talbot
J P Kellington

I. Objectives of the project:

a) To assess functional changes in alveolar macrophages (AM) induced by inhaled α-emitting actinides.

b) To study the effects of inhaled actinides on the AM pool, on AM recruitment to the lung and on AM kinetics.

c) To study the induction of nuclear aberrations in AM and their possible role in predicting the effectiveness of inhaled actinides at inducing lung tumours.

II. Objectives for the reporting period:

A. To compare the phagocytic competence of AM in control mice and in mice exposed to $^{239}PuO_2$.

B. To develop techniques for studying the cell cycle kinetics of AM.

C. To extend the work on the induction of nuclear aberrations to lower doses than used heretofore.

III. Progress achieved:

A. Effects of $^{239}PuO_2$ on the phagocytic competence of alveolar macrophages

Methods

In the progress report for 1987, we described a technique for quantifying the uptake of 1 μm diam. fluorescent latex particles (FLP) by alveolar macrophages (AM) using flow cytometry (FCM). This technique has been applied to study the uptake of FLP by AM in vivo following exposure to $^{239}PuO_2$. Mice from this group, and from a sham-exposed control group, were killed at 7, 21, 35 and 98 days following exposure to $^{239}PuO_2$ to give an IAD of 250 Bq. Two days before sacrifice, mice were exposed to airborne FLP. The lungs were lavaged with physiological saline to recover AM and the recovered cells were analysed by FCM. In addition, cytospins of AM were prepared so that the results obtained by FCM could be compared with those obtained by manual scoring of cells using a conventional microscope equipped with epifluorescence.

Results and Discussion

The results of the intercomparison showed that there was excellent agreement in estimates of the fraction of AM which contained FLP. However, estimates by FCM of the number of FLP per AM were unreliable when numbers exceeded a value of about 10. The results of measurements of labelling index of AM showed that differences between experimental and control mice were not significant at any time point (see Table). Indeed, the labelling index in experimental mice at 7 days appeared to be greater than in controls, possibly because of the reduction in AM numbers at this time. Examination of cytocentrifuge preparations showed that in experimental mice, more AM contained many (30+) FLP than in controls. Subsequent autoradiography showed that cells which contained large numbers of FLP also contained relatively large amounts of ^{239}Pu. Autoradiographic studies to quantify this correlation are in hand. Thus it appears that AM containing $^{239}PuO_2$ have an enhanced phagocytic activity.

Percent of recovered macrophages containing fluorescent particles following exposure to $^{239}PuO_2$ Mean ± SEM.

Time (d)	Control	$^{239}PuO_2$
7	49.1±10.1	55.4±4.1
22	49.9± 2.4	51.3±8.7
35	46.3±26.6	50.4±9.6
98	47.6± 4.7	46.7±0.8

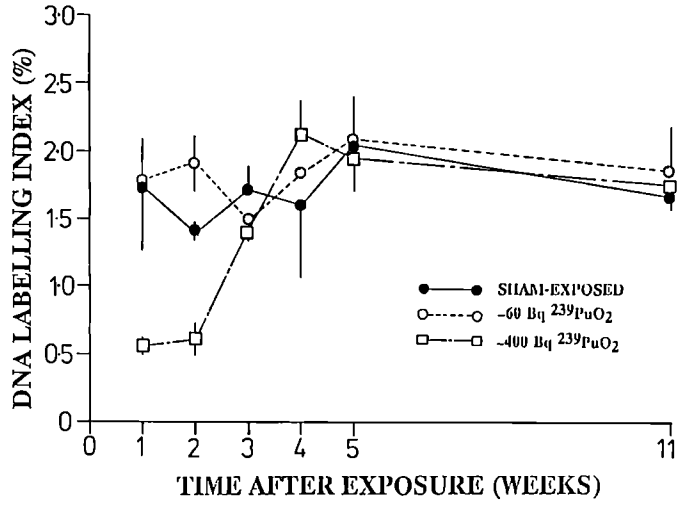
B. Effects of $^{239}PuO_2$ on alveolar macrophage kinetics

Methods

To study the kinetics of AM recruitment into the alveoli after exposure to airborne alpha-emitting actinides, it has been necessary to develop a number of new techniques. Using FCM it has been established that in normal mice more than 95% of AM are in the G1 phase of the cell cycle. A more sensitive method of identifying cells undergoing DNA synthesis was described in the previous progress report and involves the incorporation of BrdU into dividing cells. Using this technique, it has been established that the AM is not a terminally differentiated cell incapable of further division and that, under normal conditions, approximately 1.7% of the AM recovered by lavage are undergoing DNA synthesis. The effects of inhaled $^{239}PuO_2$ on AM proliferation has been studied using the BrdU technique.

Results and discussion

At early times after exposure there was a dose-dependent decrease in the number of AM undergoing DNA synthesis (see figure). This finding is consistent with previous work which demonstrated a dose-dependent decrease in the size of the AM pool and also with the concept that the AM population of the rodent lung is largely self-sustaining.



Proportion of alveolar macrophages in S-phase following exposure to $^{239}PuO_2$

C. Induction of nuclear aberrations in macrophages by $^{239}PuO_2$

A study has started to assess the yield of aberrant AM per unit dose down to an IAD of 20 Bq.

IV. Objectives for the next reporting period:

This contract terminated at the end of 1988.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None.

VI. Publications:

a) Scientific journals

Hornby S.B. and Kellington J.P. DNA synthesis in alveolar macrophages, and other changes in lavaged cells following exposure of CBA/H mice to cigarette smoke. Environ. Health Perspect. (in press).

McAnulty et al. Changes in collagen synthesis and degradation rates in the mouse lung during the development of pulmonary fibrosis induced by $^{239}PuO_2$. Clin. Sci. (in press).

Morgan et al. Induction of nuclear aberrations in mouse alveolar macrophages following exposure to $^{239}PuO_2$. A dose-response study. J. Radiol. Protect. (in press).

Oreffo V.I.C. et al. Isolation of Clara cells from the mouse lung. Environ. Health Perspect. (in press).

b) Internal report

1. McAnulty et al. Long-term changes in the mouse lung during the development of pulmonary fibrosis induced by $^{239}PuO_2$. 3. Changes in collagen synthesis and degradation rates. UKAEA Unclassified Report AERE-R 13074, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Contract no.: BI6-C-068-D

Head(s) of research team(s) [name(s) and address(es)]:

Dr. W. Schmahl
Institut für Pathologie
GSF
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Telephone number: 89-3187.2538

Title of the research contract:

Morphological and immunological characterization of cells from typical focal CNS lesions in the rat following prenatal X-irradiation and their relationships to ethylnitrosourea neurocarcinogenesis.

List of projects:

1. Morphological and immunological characterization of cells from typical focal CNS lesions in the rat following prenatal X-irradiation and their relationships to ethylnitrosourea neurocarcinogenesis.

Title of the project no.: 1

Morphological and immunological characterization of cells from typical focal CNS lesions in the rat following prenatal X-irradiation and their relationships to ethylnitrosourea neurocarcinogenesis.

Head(s) of project:

Dr. W. Schmahl

Scientific staff:

Dr. J. Plendl

I. Objectives of the project:

This project is concerned with the post-irradiational membrane properties of the neuroglioblasts in the rat fetus. These cells represent the stem cell population for the development of the central nervous system. An ordered sequence of cell division, neuron migration and settlement depends largely on functional membrane constitution, thus influencing intercellular contacts, cell communication and pattern recognition. Alterations of these membrane properties by prenatal irradiation are suggested to modify the response of single cells or of the tissue as a whole to a potential carcinogenic agent, like ethylnitrosourea (ENU).

II. Objectives for the reporting period:

The opioid system was thought to have a growth-modulating potency during central nervous system development as well as during reparation. This was demonstrated by a significant growth spurt of the neonatal brain in response to chronic naltrexone (NX) treatment during the weaning period. NX is a specific antagonist of the opioid β-endorphine at its relevant μ-receptor on both neuronal and glial cells. The growth augmentation of the brain due to NX-treatments was constantly present, even after prenatal X-irradiation with 1.0 Gy on gestation day 14. We repeated these experiments for a long-term sequential histopathological study of the forebrains, which was then completed with ^{3}H-thymidine autoradiography.

III. Progress achieved:

1. Methodology

Prenatal treatment: 16 pregnant rats were divided randomly into 2 groups. Group I remained untreated, group II animals were X-irradiated on gestation day (g.d.) 14 with 1.0 Gy.
Postnatal treatment: Pups of all litters within a given group were randomized at birth and redistributed to the nursing mothers. Litter size was kept at 10 pups to maintain uniform nutritional status. Both groups were subdivided into two halves, one without any further treatment (= a) and the other with naltrexone (NX) treatment (= b). Naltrexone was given daily by subcutaneous injection of a sterile PBS-solution of the crystalline compound (Sigma Chemie) at a dose of 50 mg/kg until postnatal day 28. The pups were weighed twice per week for adjustment of the individual dose.
Evaluation: 8 animals of either sex were randomly selected for autopsy at one of the following intervals: 4, 6, 8, 10, and 12 weeks. Four animals of either sex for each autopsy date received ^{3}H-thymidine by i.p. application, 60-70 minutes before autopsy (5/uCi/g body weight; 50-80 Ci/ mmol, New England Nuclear). Brains were embedded in paraplast and frontal serial sections (6/um) were collected for autoradiography. Labelling index of the subependymal zone (SEZ) was determined at the plane of sections Bregma 0.48 mm to - 0.80 mm according to an standard atlas of the rat brain (Bregma is defined as the point of intersection of the sagittal suture with the anterior coronal suture).

2. Results

2.1. General findings

X-irradiation exerts growth-depressing effects upon the brain which are reflected by low weights, microcephaly, hydrocephaly, hypoplasia of the subependymal stem cell layer and dystopic clusters of subependymal cells in the upper cortex layers.

2.2. Autoradiography

TABLE 1
Labelling index of the lateral subependymal zone of the rat forebrain (% ± standard error)

Treatment prenatally / postnatally		Ia 0 / 0	Ib 0 / NX	IIa 1 Gy X-irrad. / 0	IIb 1 Gy X-irrad. / NX
Age (weeks)	4	18.8 ± 0.5	18.6 ± 1.7	15.7 ± 4.6	23.2 ± 3.2
	6	15.2 ± 5.3	24.9 ± 5.3*	16.3 ± 3.4	5.9 ± 2.1*
	8	14.1 ± 2.2	20.9 ± 3.8*	9.4 ± 2.6	7.7 ± 1.3*
	10	10.9 ± 1.9	23.7 ± 4.3*	17.5 ± 4.3	19.3 ± 4.5
	12	9.5 ± 1.7	19.3 ± 4.1*	6.2 ± 2.2	11.7 ± 5.5

*significance $p < 0.005$ against controls

NX induced a persistent augmentation of the labelling frequency of the subependymal cells in the forebrain of control animals. In X-irradiated offspring the ^{3}H-thymidine incorporation was significantly depressed especially at 8 and 10 weeks of postnatal life. NX, when given to X-irradiated pups, stimulated the labelling frequency in a biphasic mode on the 4th and on the 12th week. In contrast, labelling index of 6 week old animals with X-irradiation was significantly lower after additional NX-treatment.

2.3 Histopathology

Two different forms of a hyperplastic reaction in the SEZ were observed, but only in the animals of group IIb:
2.3.1. A transitory hyperplasia of the lateral to dorsolateral districts which was confined to the period between the 4th and the 6th week and thereafter underwent regression.
2.3.2. A long-lasting hyperplasia of those SEZ districts which were dislocated due to prenatal X-irradiation. In most instances these SEZ dystopias were in the periphery of heterotopic neuronal nodules which are a typical radiation malformation of the forebrain. This hyperplastic reaction tended to change to a more dysplastic lesion in 5 of the 8 brains which were studied at 3 months. The dysplasia can be regarded as a preneoplastic state of SEZ proliferation.

3. Discussion

The chronic suppression of the endogenous opioid system in the present experiments, resulted in a twofold pattern of late effects in the forebrain of rats which were prenatally X-irradiated. The first effect, an early appearing intermittent hyperplasia of the SEZ, was beneficial for cell renewal and reparation of the radiation-induced lesion. The second finding was the long-lasting proliferation capacity of heterotopic SEZ cell clusters with a marked tendency to develop dysplasia.
These effects observed in the experimental condition give an indication of the role of the opioid system during reparation of perinatally occurring brain lesions: clearly the opioids are suppressors of cell proliferation, thus preventing reparation processes from any overshooting effects. This leads to an early extinction of the repair capacity of the rodents' forebrain in response to prenatal radiation injury. Thus the lesion type is fixed for the whole postnatal period.
This detrimental regulation was reduced by naltrexone, but this response depended whether the SEZ was in direct contact with an ependymal surface or not. In all cases of such a contact a short-lasting hyperplasia of the SEZ was found. In contrast, the dysplastic lesions only occurred when the SEZ was separated from the ependymal layer. Therefore we suppose that the ependymal cell maintains a central position in opioid-mediated growth control of the developing brain as well as in separation of radiation lesions.

IV. Objectives for the next reporting period:

1. Detailed histochemical analysis of the cells participating in the NX-induced hyperplastic lesions in forebrain of prenatally X-irradiated rats.
2. Quantitative receptor binding studies with ^{3}H-naloxone to evaluate the pharmacokinetics of the opioid system.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

FUNK, R.:
Das Wachstumsverhalten der germinativen Zone des Großhirns der Ratte nach pränataler ENU-Behandlung und postnataler Opioidrezeptorblockade.
Diss. Tierärztl. Fak., LMU München (1988)

MIASKOWSKI, U.:Das Wachstumsverhalten der germinativen Zone des Großhirns der Ratte nach pränataler Röntgenbestrahlung und postnataler Opiod-rezeptorblockade.
Diss. Tierärztl. Fak., LMU München (1988)

SCHMAHL, W.:
Schäden am Zentralnervensystem nach pränataler Strahlenexposition und deren Bedeutung für regenerative und neoplastische Prozesse.
Tagungsbericht der Schweiz. Gesellschaft für Strahlenbiologie u. Strahlenphysik 14, 41-53 (1988)

SCHMAHL, W.:
Compensative postnatal growth of radiation-induced brain dysgenesis in rats is under strong influence of the opioid system.
16th Conference on the European Teratology Society, Baveno, Italien, 18.-23.9.1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-077-D

Universitätsklinikum Essen
Institut für Medizinische
Strahlenphysik und Strahlenbiologie
Hufelandstr. 55
D - 4300 Essen 1

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. C. Streffer
Inst. Med. Strahlenphysik,-Biologie
Universitätsklinikum Essen
Hufelandstr. 55
D - 4300 Essen 1

Telephone number: 201-723 2152/53

Title of the research contract:

Investigation into biological dosimetry: Chromosomal damage and teratogenic effects following irradiation of one cell mouse embryos.

List of projects:

1. Investigation into biological dosimetry: Chromosomal damage and teratogenic effects following irradiation of one cell mouse embryos.

Title of the project no.: BI6-C-077-D

Investigation into Biological Dosimetry: Chromosomal Damage and Teratogenic Effects Following Irradiation of One Cell Mouse Embryos

Head(s) of project:

Prof. Dr. C. Streffer

Scientific staff:

Privatdozent Dr. W.-U. Müller, U. Weißenborn

I. Objectives of the project:

Exposure of different cell cycle stages of one cell mouse embryos in vivo and in vitro to different radiation qualities (neutrons, X-rays, beta-rays) and determination of:

1. chromosomal aberrations in the first, second, third, and fourth mitosis after irradiation
2. number of micronuclei in the first, second, and third interphase after irradiation
3. teratogenic effects.

Emphasis will be laid on the effects of low doses.

II. Objectives for the reporting period:

1. Frequency of chromosome aberrations in the first, second, and third mitosis after X-irradiation of one cell embryos 9 h p.c. (early G2-phase) or 12 h p.c. (late G2-phase). (h p.c. = hours post conceptionem)

2. Teratogenic effects after exposure of one cell embryos 6 h p.c. (S-phase) and 12 h p.c. (late G2-phase) to X-rays.

III. Progress achieved:

METHODOLOGY

1. Chromosome aberrations: One-cell mouse embryos were X-irradiated in vivo at various times. Shortly before the mitosis under study colchicine (1 µg per g body weight) was injected and after arresting mitosis in metaphase, embryos were flushed from the oviducts. Chromosomes were fixed using the technique of Tarkowski (Cytogenetics 5 (1966) 394).
2. Teratogenic effects were studied after X-irradiating pregnant mice at various times on day 1 of gestation. Fetuses were checked for gross abnormalities on day 19 of gestation.

RESULTS and DISCUSSION

Table 1 shows the number of chromosome aberrations in the first, second, and third mitosis after X-irradiation at different times after conception. In all cases a significant increase was observed in comparison to the control. However, the different times of irradiation revealed different sensitivities: completion of second meiotic

Table 1: Chromosome aberrations in 1st, 2nd, and 3rd mitosis after X-irradiation (0.94 Gy)
(For the sake of completeness, the results of the irradiation 1, 3, and 6 h p.c. are also quoted; these results have already been mentioned in the 1987 report)

Mitosis	Time of irrad. (h p.c.)	Chromosome type aberrations	Chromatid type aberrations	Total	No. of metaphases
1 to 2	Contr.	0.023	0	0.023	132
	1	0.167	0.034	0.210	60
	3	0.249	0.184	0.433	60
	6	0.124	0.188	0.312	64
	9	0.180	0.140	0.320	50
	12	0.129	0.519	0.648	54
2 to 4	Contr.	0.006	0.036	0.042	168
	1	0.049	0.114	0.163	61
	3	0.139	0.169	0.308	65
	6	0.125	0.145	0.270	96
	9	0.047	0.172	0.219	64
	12	0.176	0.196	0.372	51
4 to 8	Contr.	0.015	0.062	0.077	194
	1	0.086	0.100	0.186	70
	3	0.136	0.269	0.405	74
	6	0.171	0.181	0.352	88
	9	0.106	0.182	0.288	66
	12	0.186	0.228	0.414	70

division (1 h p.c.) < early G2 (9 h p.c.) < S (6 h p.c.) < G1 (3 h p.c.) < late G2 (12 h p.c.). The comparatively low sensitivity during completion of the second meiotic division may be due to the peculiar chromatin structure at this stage. The unusually low sensitivity during early G2-phase may depend either on some cells that were still in S-phase 9 h p.c. or on an effective proof-reading by repair systems shortly after DNA-reduplication.

Table 2 summarizes the results for lethal and teratogenic effects after radiation exposure of 1-cell mouse embryos. The major effect was prenatal death with G1-phase (3 h p.c.) being the most sensitive stage. Contrary to most textbook information, it was possible to induce also malformations in the 1-cell stage of our mouse strain. The type of malformation was almost exclusively a gastroschisis; in some cases exencephalies were observed. The induction of gastroschises was not restricted to the 1-cell stage, but characteristic for the entire preimplantation period (data not shown). However, the radiation doses required were higher for stages beyond the 1-cell stage.

Table 2: Prenatal death and malformations after X-irradiation of 1-cell embryos. (The data of the 1 and 3 h p.c.-irradiation, that have been reported already last year, are quoted for comparison.)

Treatment	Number of live fetuses	Number of live fetuses per mouse	Number of malformed fetuses (percentage)
Control	216	8.1	2 (0.9)
1 Gy/ 1 h p.c.	147	4.3	10* (6.8)
Control	527	8.0	6 (1.1)
1 Gy/ 3 h p.c.	343	2.9	22* (6.4)
Control	204	7.9	4 (2.0)
1 Gy/ 6 h p.c.	247	4.5	13 (5.3)
Control	200	7.7	6 (3.0)
1 Gy/12 h p.c.	251	5.3	10 (4.0)

* Significantly different from control at $P<0.01$

IV. Objectives for the next reporting period:

1. Chromosome aberrations in the first, second, and third mitosis after a 2 hr exposure of 1-cell mouse embryos to ^{3}H-thymidine or ^{3}H-arginine in vitro

2. Skeletal malformations after exposure of 1-cell embryos 3 h p.c. (G1-phase) or 12 h p.c. (late G2-phase) to 1 Gy of X-rays.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Institut für Medizinische Strahlenphysik

Leiter: Prof. Dr. rer. nat. J. Rassow

Universitätsklinikum Essen

VI. Publications:

1.
Pampfer, S.; Streffer, C.:
Prenatal death and malformations after irradiation of mouse zygotes with neutrons or X-rays.
Teratology 37 (1988) 599-607

2.
Weißenborn, U.; Streffer, C.:
Analysis of structural and numerical chromosomal anomalies at the first, second, and third mitosis after irradiation of one-cell mouse embryos with X-rays or neutrons.
Int. J. Radiat. Biol. 54 (1988) 381-394

3.
Weißenborn, U.; Streffer, C.:
The one-cell mouse embryo: cell cycle-dependent radiosensitivity and development of chromosomal anomalies in postradiation cell cycles.
Int. J. Radiat. Biol. 54 (1988) 659-674

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-C-079-NL

Division for Health Research
Radiobiological Institute TNO
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. D.W. van Bekkum
Division of Health Research
Radiobiological Institute TNO
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

Telephone number: 015-136940

Title of the research contract:

Development of conditions allowing restoration of hemopoiesis by allogenic purified and in vitro multiplied pluripotent hemopoietic stem cells.

List of projects:

1. In vitro multiplication of purified rhesus monkey and human pluripotent hemopoietic stem cells.
2. Non-lethal multi-modality conditioning for transplantation of T-lymphocyte depleted stem cell fractions.

Title of the project no.:

1. In vitro multiplication of purified rhesus monkey and human pluripotent hemopoietic stem cells.

Head(s) of project:

Dr. G. Wagemaker

Scientific staff:

Prof. Dr. D.W. van Bekkum, Dr. J.J. Wielenga, Dr. F. van Gils

I. Objectives of the project:

The project is directed at purification of rhesus monkey and human stem cells and their subsequent in vitro multiplication to achieve sufficient numbers of stem cells for sustained engraftment using partially or completely mismatched donors.

II. Objectives for the reporting period:

In agreement with the progress report 1987 the following aims were set for the reporting period:

1. molecular cloning of the gene coding rhesus monkey IL-3;
2. cell separation and stem cell purification experiments in search for accessory cells influencing the IL-3 response
3. scale expansion of stem cell purification based on binding of the cells to immunomagnetic beads using a monoclonal antibody against HPCA-1

III. Progress achieved:

The molecular cloning of the gene encoding rhesus monkey IL-3 had been accomplished. The nucleotide sequence of the rhesus monkey IL-3 gene compared with the human IL-3 gene displays more than 90% homology and is similar to the human gene, divided into five small exons; the mature protein comprises 124 amino acids, which is 9 residues shorter than the human counterpart. The two cystein residues that play an essential role in protein folding in the murine as well as the human IL-3 are also conserved in the rhesus IL-3. The rhesus IL-3 gene expressed in COS-cells yielded a product that was biologically active using rhesus as well as human bone marrow cells as targets, although the human IL-3 so far was inactive for rhesus monkey cells. This peculiar species specificity pattern, which might be caused by glycosylation differences between the preparations used, is subject to further study, which will be carried out in conjunction with the establishment of an efficient production line for rhesus IL-3 using expression in various micro-organisms. The resulting preparation will be used for detailed studies on stem cell replication using highly purified stem cell prerations described below in synergy with other hemopoietic growth factors as reported earlier.

In humans it became established that the full expression of the stimulatory effects of IL-3 is dependent on accessory cells belonging to the monocyte lineage. Most likely, early multipotent hemopoietic progenitor cells (CFU-mix, BFU-E) respond directly to IL-3, whereas more mature progenitor cells (CFU-G, CFU-GM, and CFU-M) require factors produced by these accessory cells. These observations confirm a restricted direct effect of IL-3 on immature cells, probably including stem cells. A more detailed analysis using the rhesus monkey is dependent on the development of an efficient production line for rhesus IL-3 and scheduled for 1989.

The development of a large scale purification method for rhesus monkey stem cells based on the surface antigen CD34 using immunomagnetic beads has been extensively studied in the reporting period. Using a combination of density gradient centrifugation, T-lymphocyte depletion and binding to immunomagnetic beads, an approximately 100- to 200-fold enrichment can be achieved with a recovery of approximately 60%, based on GM-CFU counts. Experiments are scheduled for 1989 to test the repopulating capacity of these enriched preparations by transplantation in autologous as well as allogeneic rhesus monkey recipients.

IV. Objectives for the next reporting period:

In the next reporting period this project will be extended along the lines reported under III.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Primate Center TNO, P.O.Box 5815, 2280 HV Rijswijk, The Netherlands
Institute for Experimental Gerontology, P.O.Box 5815, 2280 HV Rijswijk, The Netherlands
Dept. of Radiobiology, Erasmus University, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands
Dr. Daniël den Hoedkliniek, P.O.Box 5201, 3008 AE Rotterdam, The Netherlands.

VI. Publications:

Included in the publication list of project 2.

Title of the project no.:

2. Non-lethal multi-modality conditioning for transplantation of T-lymphocyte depleted stem cell fractions.

Head(s) of project:

Prof. Dr. D.W. van Bekkum

Scientific staff:

Dr. J.J. Wielenga, Dr. P.J. Heidt, Dr. G. Wagemaker, Dr. J. Zoetelief

I. Objectives of the project:

This project is devoted to the development of nonlethal conditioning regimens as an adjuvant to a moderately high dose of total body irradiation, including the isolation, characterisation and production of a lymphokine that suppresses the action of T-lymphocytes.

II. Objectives of the reporting period:

In agreement with the progress report 1987 the following aims were set for the reporting period:

1. Tests with immunosuppressive monoclonal antibodies as adjuvants to total body irradiation for bone marrow transplantation in mice;
2. Tests of the immunosuppressive monoclonal anti-human LFA-1 for bone marrow transplantation in rhesus monkeys;
3. Development of a method to purify and produce the lymphokine that suppresses the action of T-lymphocytes

III. Progress achieved:

Monoclonal antibodies directed against T-lymphocytes are thought to be the most promising nontoxic immunosuppressive agents for conditioning for bone marrow transplantation in victims of radiation accidents. The monoclonal antibodies currently tested in mice include rat-anti-mouse L3T4/Lyt-2 (CD4/CD8) monoclonal antibodies of IgG2b isotype (Department of Pathology, Cambridge University, UK) and rat-anti-mouse Thy-1 of IgG2b isotype (Department of Immunology, Institute of Hematology, GSF, Munich, FRG). Currently, we are testing these monoclonals in semi-allogeneic and allogeneic mouse W/W^{v} models for partial chimerism, notably alpha-thalassemic mice and W/W^{v} mice. The former (Wagemaker et al., Transplantation 42, 1986, 248 -251) are an optimal model to test the immunosuppressive potential of monoclonal antibodies, since engraftment can be longitudinally followed in single mice by means of the red cell size marker. Using these mice, it was shown that the anti L3T4/Lyt2 combination of monoclonal antibodies have an immunosuppressive capacity equivalent tot 3 to 4 Gy TBI, while that of anti-Thy-1 is comparable to 2 to 3 Gy TBI. The experiments will be concluded in 1989 to reach optimal time and dosage scheduling, and will be extended for possible synergism between monoclonals directed aginst different antigens, including anti-mouse LFA-1, and with other immunosuppressive agents.

Anti-human LFA-1 was found to cross-react with rhesus monkey cells. Based on the hypothesis that graft rejection has similar effector cells as graft-versus-host reactions, the immunosuppressive effectiveness of anti-LFA-1 was tested in rhesus monkeys in a graft-versus-host model (a fully mismatched donor-recipient combination) as well as a host-versus-graft model (8.5 Gy X-rays as conditioning, using a 3-4 log T lymphocyte depleted, fully mismatched bone marrow graft). Anti-LFA-1 appeared to be incapable to prevent lethal graft-versus-host disease or graft rejection, respectively, and it is concluded that its immuno-suppressive action as a single agent is weak. The experiments will be extended in 1989 if the mouse experiments reveal synergism of anti-LFA-1 and other monoclonals against T lymphocytes.

Research on the suppressor factor (SUF) prepared from the large molecular weight factor of the supernatant of hybridoma cells has been continued. The Graft-versus-Host reaction induced by allogeneic spleen cells is prevented by incubated spleen cells with SUF prior to grafting. It has been found that SUF is also capable of preventing the capacity of isogeneic spleen cells to reject an allogeneic bone marrow graft indicating that graft rejection is due to immunological reaction by T-lymphocytes. The high molecular weight SUF has been studied in vitro on T-lymphocyte populations. It prevents the expression of the IL-2 receptor on stimulated T-cells, but does not affect the utilisation of IL-2 by cells expressing IL-2 receptors. Purification of SUF is proceeding with a variety of methods. So far, a purification factor of 2500 times has been achieved. Following further purification, molecular cloning and production of bulk SUF by recombinant DNA-techniques will be undertaken.

IV. Objectives for the next reporting period:

In the next reporting period this project will be extended along the lines reported under III.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Primate Center TNO, P.O.Box 5815, 2280 HV Rijswijk, The Netherlands
Institute for Experimental Gerontology, P.O.Box 5815, 2280 HV Rijswijk, The Netherlands
Dept. of Radiobiology, Erasmus University, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.

VI. Publications:

Gerritsen WR, Wagemaker G, Jonker M, Kenter MJ, Wielenga JJ, Hale G, Waldmann H, Van Bekkum DW.
The repopulation capacity of bone marrow grafts following pretreatment with monoclonal antibodies against T lymphocytes in rhesus monkeys. Transplantation 1988;45,2;301-307.

Hoogerbrugge PM, Suzuku Kinuko, Suzuki Kunihiko, Poorthuis BJHM, Kobayashi T, Wagemaker G, Van Bekkum DW.
Donor-derived cells in the central nervous system of twitcher mice after bone marrow transplantation. Science 1988;239,4843;1035-1038.

Mulder AH, Visser JWM, Zoetelief J, Van Bekkum DW.
The entry of the prothymocyte into the thymus after lethal irradiation and bone marrow transplantation. II. Time of entry. Thymus 1988;11,1; 29-41.

Suzuki Kinuko, Hoogerbrugge PM, Poorthuis BJHM, Van Bekkum DW, Suzuki Kunihiko.
The twitcher mouse: Central nervous pathology after bone marrow transplantation. Lab. Invest. 1988;58,3;302-309.

Bot FJ, Dorssers L, Wagemaker G, Löwenberg B.
Stimulating spectrum of human recombinant multi-CSF (IL-3) on human marrow precursors: importance of accessory cells. Blood 1988;71,6; 1609-1614.

Heidt PJ.
Management of bacterial and fungal infections in bone marrow transplant recipients and other granulocytopenic patients. Cancer Detect. Prevention 12, 1988, 609-619.

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Effect of bone marrow transplantation on enzyme levels and clinical course in the neurologically affected twitcher mouse. Journal Clinical Investigation 1988;81;1790-1794.

Zoetelief J, De Wit NJP, Wielenga JJ, Wagemaker G, Broerse JJ.
Dosimetry for whole-body irradiations of monkeys with 300 kV X-rays. Radiat. Biol. 1988;54,5;862-863.

Delwel R, Salem M, Pellens C, Dorssers L, Wagemaker G, Clark S, Löwenberg B.
Growth regulation of human acute myeloid leukemia: effects of five recombinant hematopoietic factors in a serum-free culture system. Blood 1988;72,6;1944-1949.

Van Bekkum DW.
The acquired immunedeficiency syndrome in man. In: New developments in biosciences their implications for laboratory animal science. Proc. of the Third Symposium of the Federation of European Laboratory Animal Science Associations, Amsterdam, June 1-5, 1987. A.C. Beynen and H.A. Solleveld (eds), Dordrecht, M. Nijhoff, 1988; 7-10.

Broerse JJ, Zoetelief J, Van Bekkum DW.
LD-50 in man and monkeys and the effect of inhomogeneous dose distribution. In: Proceedings of the 1987 workshop of the Research Study Group on the Assessment of Ionizing Radiation Injury in Nuclear Warfare, Alverstoke, Gosport, U.K., May 1987, NATO Defence Research Group, 1988; 6-21.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Rue Charles Lemaire, 1
B - 1160 Bruxelles

Contract no.: BI6-C-081-B

Head(s) of research team(s) [name(s) and address(es)]:

Dr. O. Vanderborght
Département de Radiobiologie
CEN/SCK
Boeretang 200
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Telephone number: 014-31.18.01

Title of the research contract:

Comparison of damage from internal alpha irradiation to the hemopoietic and stromal system in adult and pre- and postnatal animals.

List of projects:

1. Comparison of damage from internal alpha irradiation to the hemopoietic and stromal system in adult and pre- and postnatal animals.

Title of the project no.:

Comparison of damage from internal alpha-irradiation on the hemopoietic and stromal system in adult and pre- and postnatal mice.

Head(s) of project:

Greet E.R. Schoeters

Scientific staff:

R. Van Den Heuvel
G. Schoeters
O. Vanderborght

I. Objectives of the project:

This study aims at elucidating cells at risk with respect to the induction of long-term effects like bone tumors and myeloproliferative disorders. Effects on stromal and hemopoietic stem cells of hemopoietic organs are studied after contamination of mice with ^{241}Am. The investigation includes different age groups : foetal, neonatal and adult mice. Their sensitivity to internal alpha-emitters will be compared.

II. Objectives for the reporting period:

1. A survival experiment using offsprings of ^{241}Am contaminated pregnant mice which were reared by a fostermother is continued. The occurence of late radiation effects is checked.
2. The accumulated alpha-dose at which foetal marrow cells are affected after ^{241}Am injection in utero at day 14 of pregnancy is 30-40 times smaller than the lowest dose at which changes have been noticed after injection of adult mice (evaluated in long-term bone marrow cultures = LTC).
   - Does this radiation damage to marrow cells persist 71 weeks postcontamination ?
   - Which are the radiosensitive targets in the marrow which are responsible for the observed damage in these LTC ?
3. To develop further the in vitro assay for growth of osteoprogenitor cells out of adult bone marrow.
4. Study of the stromal contribution for haemopoiesis in long-term cultures of liver, spleen and bone marrow of pre- and postnatal mice.

III. Progress achieved:

Methodology

1. Pregnant BALB/c mice at 14 days of gestation were injected with 0, 100, 500 and 1500 kBq ^{241}Am/kg. At birth, newborn mice are transferred to a fostermother. At 3 weeks of age, mice are weaned and housed individually.
2. Long-term marrow cultures from offsprings of contaminated pregnant mice (0, 3.35, 13.35, 22.6, 49.4 kBq ^{241}Am/mouse) are initiated at 6, 17 and 71 weeks postcontamination.

a. The CFU-GM proliferation is checked in these cultures.

b. An experiment was designed to test which component in the long-term cultures, either the stromal adherent layer or the hemopoietic stem cells, is radiosensitive. Stromal layers in LTC from contaminated and non-contaminated offsprings, were irradiated (10 Gy X-irradiation) or grown in 25% FCS (two methods which make hemopoietic stem cells disappear to get a purified stromal layer). After depletion of haemopoietic stem cells, cultures were loaded with fresh bone marrow from non-contaminated or ^{241}Am contaminated mice (to compare the capacities of the stromas and to compare the quality of the haemopoietic cell population). Several weeks after recharging, CFU-GM production in the LTC was followed.

c. CFU-s, CFU-GM and CFU-f stem cell assays were performed from the bone marrow of the offsprings.

3. Marrow from femurs of adult BALB/c mice was asceptically collected as a marrow plug without disrupting its original three-dimensional structure. Tissue culture medium consisted of BGJB medium supplemented with 10% foetal calf serum, 1% L-glutamine, 1% gentamicine, 10^{-2} M beta-glycerophosphate and l-ascorbic acid. The cultures were incubated at 37°C in 5% CO_2 (90% relative humidity). Enzyme histochemistry, transmission electron microscopy and labeling of cultures with radioactive compounds, ^{3}H-proline, ^{3}H-thymidine, ^{85}Sr were used to display characteristics of the new model.
4. The CFU-GM yield in confluent long-term cultures derived from liver, spleen and bone marrow cells at different gestational and postnatal ages has been studied.

Results

1. This survival experiment is going on. Up to now, 193 of 579 mice died. Histological examinations were carried out. Results are not complete yet.
2. a. Long-term bone marrow cultures from offsprings contaminated in utero were less able to support CFU-GM proliferation than control LTC from non-contaminated offsprings. This radiation damage persisted 71 weeks after contamination in utero.

   b. No differences (at 6 weeks postcontamination) were seen between the stromal layers of control mice or contaminated mice. The stromal layers from control mice and contaminated mice were as effective in supporting control haemopoietic cells and haemopoietic cells from contaminated mice. No differences were seen between the haemopoietic cell suspensions from control or contaminated mice brought on identical stromal layers, on the one hand control stromal layers and on the other stromal layers derived from contaminated mice. At 17 weeks postcontamination, results of this exchange experiment are not unambiguous.

c. The haemopoietic stem cell concentration (CFU-s, CFU-GM) and the stromal stem cell concentration (CFU-f) in the bone marrow from which LTC were initiated and which were used to reload the cultures, were not impaired after ^{241}Am contamination in utero.

3. Murine adult bone marrow cells exhibit a mineralizing capacity in vitro. In less than 2 weeks after the onset of the cultures, mineralization is obtained in more than 80% of the marrow cultures. Morphological studies reveal that during incubation phenotype changes related to osteogenic differentiation occur at the extracellular matrix as well as at cell populations. Well banded collagen is synthetized. Matrix vesicles and needles of hydroxy-apatite crystals are observed via transmission electron microscopy. Osteoblastlike cells are present with membrane associated alkaline phosphatase activity. The mineralization is specific for cultured bone marrow and is not observed in cultured spleen fragments as is shown via ^{85}Sr uptake, calcein uptake and histomorphology.
4. The stromal cell compartment of fetal and neonatal haemopoietic organs is able to sustain haemopoiesis in vitro. Moreover, the granulocyte-macrophage stem cell (CFU-GM) yield of these LTC reflects the CFU-GM content of the haemopoietic organs from which the cultures are originated. LTC from the liver produce high numbers of CFU-GM if the cultures are derived from fetal livers between 13 d of gestation and birth. Cultures from spleens just before and after birth, give maximal CFU-GM numbers. The CFU-GM yield in long-term bone marrow cultures increases 10 times from 17 day old fetus towards adult life.

Discussion

1. The occurence of late radiation effects will be checked.
2. Radiation damage in the LTC is still present a long time after contamination (71 weeks). This late effect is very important. This implicates that foetal and neonatal animals are very radiosensitive because low accumulated alpha-radiation doses cause damage to the bone marrow and this damage is not repaired 1.5 year after contamination (cumulative dose to the femur at 71 weeks postcontamination is 0.065 Gy).
   The damage we see in LTC derived from mice contaminated in utero cannot be explained by a diminished marrow cellularity or differences in stem cell concentrations.
   Question to be explored further remains : what is the radiosensitive component in the LTC (cell-cell interaction, extracellular matrix synthesis, growth factor production) ?
   Our results indicate that perinatal bone marrow cells are very radiosensitive and can be considered as target cells for early changes and late effects after contamination in utero. Besides, these observations are important for risk estimations concerning pregnant animals and neonates. Moreover, the long-term bone marrow culture technique is a very sensitive system to examine radiation damage.
3. The in vitro organ culture we developed may provide the opportunity to identify which marrow cells have osteogenic potential and to investigate the mechanisms triggering differentiation towards osteogenesis.
4. LTC present an important advance in our ability to study the role of interactions between stromal cells and haemopoietic stem cells. The LTC system of fetal haemopoietic organs is a useful technique for the analysis of factors responsible for changes in the haemopoietic microenvironment during fetal development and is an experimental model for the study of the role of the fetal stromal microenvironment in the migration of haemopoiesis to other

IV. Objectives for the next reporting period:

1. The long-term experiment will be continued : survival data will be collected, soft tissue histology will go on and post-mortem radiographs will be made from the skeleton.
2. The assay for in vitro mineralization will be worked out further. The radiosensitivity of the assay, and its applicability for research on effects of bone-seeking radionuclides will be tested. This technique can be used as a model for osteogenic differentiation which in turn helps to elucidate the mechanisms of osteosarcoma induction and bone diseases.
3. The capacity to maintain CFU-GM proliferation is affected in LTC derived from mice contaminated with ^{241}Am in utero. To determine wich components (stromal cells or haemopoietic cells) are important for in vitro haemopoiesis we perform exchange experiments between stromal adherent livers and haemopoietic cell suspensions derived from active and non-active haemopoietic liver, spleen and bone marrow.

V. Other research group(s) collaborating actively on this project (names and address(es))

Dr. B. Lord, Radium Holt Institute, Manchester, U.K.
Dr. J. Schmidt, Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, West Germany

VI. Publications:

R. Van Den Heuvel, G. Schoeters, O. Vanderborght
Increased efficiency of CFU-GM production after miniaturization of long-term murine bone marrow cultures.
Archives of Biology 99, 157-167 (1988).

R.L. Van Den Heuvel, G.E.R. Schoeters, O.L.J. Vanderborght
Haemopoiesis in long-term cultures of liver, spleen and bone marrow of pre- and postnatal mice : CFU-GM production.
British Journal of Haematology 70, 273-277 (1988).

G. Schoeters, B. Lord
Stem cells in bone and bone marrow after contamination with bone-seeking radionuclides.
Workshop report, Antwerp, Belgium, 29-30 September 1987. International Journal of Radiation Biology 53, 691-695 (1988).

G. Schoeters, R. Van Den Heuvel
The distribution of ^{241}Am in the mouse fetus and its effects.
EULEP Newsletter 50, 22-23 (1988).

G. Schoeters, L. de Saint-Georges, R. Van Den Heuvel, O. Vanderborght
Mineralization of adult mouse bone marrow in vitro. Cell and Tissue Kinetics (accepted).

III D

STRAHLENKARZINOGENESE

RADIATION CARCINOGENESIS

RADIOCANCEROGENESE

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-D-064-UK

Head(s) of research team(s) [name(s) and address(es)]:

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MRC
Harwell, Didcot
GB - Oxon OX11 ORD

Telephone number: 0235-834393

Title of the research contract:

Studies on myeloid leukaemia and osteosarcoma induced in mice by Ra-224.

List of projects:

1. Ratios of yields of myeloid leukaemia and osteosarcoma induced in mice by Ra-224.
2. The role of oncogene activation in Ra-224 induced myeloid leukaemia.

Title of the project no.: 1

Ratios of yields of myeloid leukaemia and osteosarcoma induced in mice by ^{224}Ra.

Head(s) of project:

E.R.Humphreys

Scientific staff:

E.R.Humphreys

I. Objectives of the project:

To show, in CBA/H mice, that the yield of myeloid leukaemia is greater than that of osteosarcoma following the injection of amounts of ^{224}Ra which are less than optimum for inducing osteosarcoma.

II. Objectives for the reporting period:

1. To continue the investigation of a single injection of different amounts of ^{224}Ra into male CBA/H mice and to begin the identification of subtypes of the induced myeloid leukaemias by cell morphology.

2. To begin a long-term investigation of the effects in male CBA/H mice of the protraction in time of the administration of ^{224}Ra

III. Progress achieved:

1. THE SINGLE INJECTION EXPERIMENT

1.1 METHODOLOGY

(See previous reports)

1.2 RESULTS

Table 1 shows the present (December 1988) status of the single injection experiment. The unequal rates of death in the five groups can be attributed to a greater initial rate of introduction of mice into groups 3 and 4 than into the other three groups. The differences in yields of myeloid leukaemia and of osteosarcoma are, to some extent, also a reflection of this.

Table 1
Single injection experiment
Status December 1988

Group	1	2	3	4	5
^{224}Ra injected (Bq g^{-1})	0	69	138	280	555
No. of mice entered	400	400	400	400	400
No. of mice dead	133	149	300	310	90
Mice diagnosed as having:					
Myeloid leukaemia	0	1	11	16	4
Osteosarcoma	0	1	2	2	0

Table 2 shows the measurements made in the myeloid leukaemic mice. Only one of these animals died before provisional diagnosis (in group 2); all the remaining leukaemias were successfully transplanted and were diagnosable in the chimaeras after an average time of about 50 days.

Table 2
Single injection experiment
Mean values (±SEM) in myeloid leukaemia mice

Group	2	3	4	5
^{224}Ra (Bqg^{-1})	68.4	137±3	273±6	598±42
No. of mice	1	11	16	4
Mean days inj.-death	361	467±43	566±30	462±49
Spleen mass (mg)	-	717±53	612±35	413±108
WBC (mm^{-3} X 10^{-3})	-	69±17	29±4	48±22
RBC (mm^{-3} X 10^{-6})	-	5.2±0.5	6.2±0.4	4.5±0.7

A concurrent study of 21 of the induced myeloid leukaemias by blood cell morphology has shown a tentative similarity with four of the seven subtypes of human acute myeloid leukaemia listed in the FAB classification of human acute non-lymphocytic leukaemias.

1.3 DISCUSSION

The results continue to show that the relationship between the yield of myeloid leukaemia and the amount of ^{224}Ra injected is curvilinear. When the yields of myeloid leukaemia are expressed in terms of mouse days exposure (therefore taking competing causes of death into account), the yields are 0.54, 4.08, 5.93 and 2.86 myeloid leukaemias per 100000 mouse days exposure in groups 2, 3, 4 and 5 respectively. Overall, the ratio of myeloid leukaemia to osteosarcoma induction is 6.4. The influence of the different rates of introduction of animals into the different groups on both of these relationships will diminish as the experiment nears completion.

2.THE MULTIPLE INJECTION EXPERIMENT

2.1 METHODOLOGY

Three groups of 200 12 week old male CBA/H mice have been injected with total amounts of 32, 64 and 128 Bq g^{-1} ^{224}Ra in twice weekly equal aliquots over a period of eight weeks. All injections were made intraperitoneally as a solution of the nuclide in physiological saline containing

100µg cm^{-3} Ca^{2+}. The subsequent housing and treatment of the animals is the same as that which has been described for the single injection experiments (see previous reports) with the exception that radiographs are not taken routinely. Group 3 (128Bq g^{-1}) was injected between January and March 1988 and groups 1 and 2 between July and October 1988.

2.2 RESULTS

The setup of the experiment and the results to date (December 1988) are shown in table 3.

Table 3
Multiple injection experiment
Status December 1988

Group	1	2	3
^{224}Ra injected (Bq g^{-1})	32	64	128
No. of mice entered	200	200	200
No. of mice dead	5	8	15
Mice diagnosed as having:			
Myeloid leukaemia	1	0	2

The periods between first injection and diagnosis of myeloid leukaemia are 77 days for the single incidence in group 1 and 15 and 87 days for the two leukaemias in group 3. The myeloid leukaemias in group 3 have been diagnosed provisionally as erythrocytic.

DISCUSSION

The experiment is not sufficiently advanced to draw any firm conclusions about the effects of protracted administration of ^{224}Ra. Nevertheless the early induction of myeloid leukaemias, although small, are similar to the findings in Neuherberg (Muller et al Health Physics 54 461 1988). The diagnoses of the erythrocytic sub-types were unexpected and raise questions about the locations of specific precursors in the marrow.

IV. Objectives for the next reporting period:

To continue observations, collection and analysis of data from the experiments in which ^{224}Ra has been administered to male CBA/H mice in single and multiple injection.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Humphreys,E.R. (1988)
Is a dose/response relationship a valid concept for the induction of leukaemia by bone-seeking α-emitting radionuclides?
14th L.H.Gray Conference, New College, Oxford, United Kingdom 11 - 15 September

Humphreys,E.R. and Humm,J.L. (1988)
A Monte-Carlo approach to the microdosimetry of ^{224}Ra in murine compact and cancellous bone.
Health Physics 54 607 - 615

Humphreys,E.R., Major,I.R. and Stones,V.A. (1988)
Myeloid leukaemia/osteosarcoma ratio in CBA/H mice given ^{224}Ra - interim results.
British Journal of Radiology In Press

Title of the project no.: 2

The role of oncogene activation in ^{224}Ra-induced myeloid leukaemia.

Head(s) of project:

Dr. R. Cox Prof. G.E. Adams

Scientific staff:

Dr. E. R. Humphreys

I. Objectives of the project:

To determine the involvement of specific oncogene activation events in the induction of murine myeloid leukaemia by the bone-seeking radio-nuclide ^{224}Ra and whether such activation events are linked with radiation-induced chromosomal changes in target bone-marrow cells.

II. Objectives for the reporting period:

1. To continue karyotypic analyses of radiation induced AMLs, derivative cell lines and somatic cell hybrids.

2. To continue studies on clonal proliferation following α-particle irradiation and transplantion.

3. To continue studies on DNA structure and mRNA expression of proto-oncogene and growth factor gene sequences.

III. Progress achieved:

1. Methodology

1.1. Karyotypic analyses: The karyotypes of AMLs, derivative cell lines and somatic cell hybrids were analysed using G-banding techniques. In situ hybridization of ^{3}H labelled gene probes to metaphase chromosomes was achieved using published techniques (Human Genetic Diseases, Davies K.(ed) IRL Press 1987 p 85).

1.2. In vitro cellular irradiation and transplantation The clonal proliferation of in vitro α-particle irradiated (~1.3Gy) multipotential haemopoietic cells was studied by karyotypic analysis of the re-populating haemopoietic systems of transplanted recipient animals.

1.3. Molecular analyses of genes in normal and leukaemic cells High MWT DNA and mRNA were extracted from cells and subjected to standard electrophoretic and molecular hybridization analyses. Pulse field gel electrophoretic (PFGE) analysis of DNA has been developed according to published outlines (Trends Genet. 3:167 1987).

2. Results

2.1. Karyotypic analyses: An image processing system for murine karyotypic analysis is now operational and functioning with an accuracy that usually exceeds two assignment error per metaphase (ref 3). Further X-ray and α-particle induced AMLs have been characterised. Many carry the characteristic chomosome (ch)2 rearrangement and will provide an additional resource for subsequent molecular analysis. From an initial large series, three somatic cell hybrid clones segregating normal and rearranged ch2 copies have been obtained and shown by molecular analyses to contain ch2 encoded sequences.

2.2. In vitro cellular irradiation and transplantation: In vitro α-particles irradiated haemopoietic cells repopulating marrow-ablated recipients gave rise to relatively few cell clones carrying stable chromosomal changes - the majority of repopulating cells had normal karyotypes. In particular, the frequency of ch2-rearranged cell clones was dramatically less than that previously observed after a 3Gy dose of X-rays (see previous reports).

Molecular analyses of genes in normal and leukaemic cells: Following negative results with other ch2-encoded genes attention has been focussed on the cytokine genes interleukin(IL)-1α and β recently assigned to ch2. In situ hybridization techniques were used to locate IL-1α and β to the F region of normal ch2 copies, close to a frequent breakpoint seen in AMLs. In an AML (N36) carrying a ch2 -> 2 translocation one IL-1β copy was shown to be translocated and it was provisionally concluded that it was located close to a C2/F translocation junction. Analysis of IL-1β mRNA in normal marrow, AML N36 and two other ch2 rearranged AMLs showed that normal sized (1.8 kb) IL-1β transcripts were substantially increased in abundance in the AMLs. In contrast, no significant changes were seen in the mRNA abundance of IL-1α nor of the growth factors GMCSF, CSF-1 and IL-3. A direct association between ch2 rearrangement and IL-1β deregulation was further supported by the observation of sub-normal levels of IL-1β mRNA in an AML lacking ch2 rearrangement. Conventional DNA analyses provided no evidence of intragenic IL-1α or β rearrangement in AMLs but recent PFGE studies indicate the presence of a possible IL-1β flanking sequence rearrangement in AML N36. This may correspond to the C2/F translocation junction that appears to characterise this neoplasm.

Discussion

The low efficiency with which α-particles induce stable chromosomal changes in multipotential haemopoietic cells probably derives from the inherent sensitivity of these cells to an α-particle traversal of the nucleus. If such a traversal usually results in a level of genomic damage that is incompatible with clonal survival then the majority of repopulating cells will be those that did not sustain a direct α-traversal of the nucleus and therefore will not show a high level of chromosomal rearrangement. If correct, this explanation may have important implications for leukaemogenesis by bone-seeking α-particle emitting radionuclides. The association that we have established between murine ch2 rearrangement in X-irradiated multipotential haemopoietic cells and in overt AMLs supports the contention that these events may initiate radiation leukaemogenesis. Molecular studies now show that specific forms of ch2 rearrangement in AMLs appear to be causually linked with deregulation of the cytokine gene IL-1β. Further studies are in progress to confirm this. IL-1 peptides are known to target early haemopoietic cells, probably via the stimulation of growth factor production by stromal cells. Deregulation of IL-1β in ch2 rearranged multipotential cells may therefore trigger a persistent, locally acting proliferative stimulus. Such uncoupling of normal growth control, while not overtly oncogenic, may generate unusually rapid and extensive clonal expansion of IL-1 deregulated cells and increase the probability of the clonal accumulation of secondary events leading to overt myeloid malignancy. Deregulation of IL-1β is a frequent finding in human AML and, in the mouse, appears to be a strong candidate for a radiation-induced initiating event for myeloid leukaemogenesis.

IV. Objectives for the next reporting period:

1. To continue karyotypic analyses of radiation-induced AMLs derivative cells lines and somatic cell hybrids.

2. To continue studies on clonal proliferation following X- or α particle irradiation and transplantation.

3. To continue studies on DNA structure and mRNA expression of proto-oncogene and growth factor gene sequences with particular emphasis on IL-1β.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs. J. Piper and D. Rutovitz, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, U.K.

Dr. A.R. Shaw, Glaxo Institute for Molecular Biology, 1211 Geneva, Switzerland.

Drs. E.G. Wright and D.T. Goodhead, MRC Radiobiology Unit, Chilton, Didcot, Oxon, U.K.

VI. Publications:

1. SILVER, A.R.J., MASSON, W.K., BRECKON, G., COX, R. (1988) Preliminary molecular studies on two chromosome 2 encoded genes c-abl and β2m in radiation-induced murine myeloid leukaemias. Int. J. Radiat. Bio. 53:57-63.

2. BRECKON, G., SILVER, A.R.J., COX, R. (1988) Consistent chromosome changes in radiation-induced murine leukaemias. In Brandham, P.E. and Bennett, M.D. (ed) Kew Chromosome Conference III HMSO p 179.

3. PIPER, J. AND BRECKON, G. An automated system for karyotyping mouse chromosomes. Cytogenetics and Cell Genetics (in the press).

4. SILVER, A.R.J., BRECKON, G., BOULTWOOD, J., ADAM, J., MASSON, W.K., COX, R. Studies on putative intiating events for radiation oncogenesis. Int. J. Radiat. Biol. (in the press).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-067-NL

Radiobiological Institute TNO
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Lange Kleiweg, 151
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Telephone number: 015-136940

Title of the research contract:

Relative biological effectiveness for the induction of malignant characteristics in cells by fast neutrons and of lung cancer by radon.

List of projects:

1. Measurements of the biological effectiveness of different types of radiation for induction of chromosome damage and cell reproductive death.
2. Transformation of cells in culture by ionizing radiations of different linear energy transfer.
3. Experimental studies on lung tumour induction by inhalation of radon in combination with some promoting agents, present in cigarette smoke.

Title of the project no.: I

Measurements of the biological effectiveness of different types of radiation for induction of chromosome damage and cell reproductive death.

Head(s) of project:

Prof.dr. G.W. Barendsen

Scientific staff:

G.W. Barendsen, J. Zoetelief, H.B. Kal, J.J. Broerse

I. Objectives of the project:

The objective of this project is to obtain insight in shapes of dose-response relationships and mechanisms by which various types of effects are induced in mammalian cells by ionizing radiations of different linear energy transfer. An analysis with respect to similarities and differences in various parameters of the dose-effect relations and of RBE values will be performed to assess implications for hypotheses about mechanisms involving the induction of effects by single tracks of particles and by accumulation of damage from different tracks. Possible differences in characteristic parameters are presumably related to differences in repair of damage in cells. Chromosome damage is at least in part the cause of cell reproductive death, but can also cause other changes expressed in surviving cells. A better understanding of these effects will contribute to a basis for assessment of Q-values for radiation protection.

II. Objectives for the reporting period:

Studies will be performed to analyse dose-effect relationships for the induction of chromosome damage and cell reproductive death in mammalian cells irradiated with photons and neutrons. To apply modern flow cytometry to the study of radiation induced cellular damage, a high resolution apparatus for flow karyometry has been built, which has to be optimalized for the detection of chromosome aberrations.

III. Progress achieved:

Measurements of chromosome aberrations by flow karyometry have been continued with the aim to improve the methods by which reproducible two parameter karyograms can be obtained for a variety of cell types, including diploid cells of human origin.
The shift of emphasis to studies of human cells is motivated by the fact that the practical application in biological dosimetry will involve human cells which have a much larger number of 46 chromosomes as compare with the 22 chromosomes of the NBCH cells of Chinese hamster origin. As a consequence of the large number of chromosomes double labelling with Hoechst and Chromomycin is required and the analysis of radiation induced changes in these karyograms is more complicated. High resolution and good reproducibility are required to quantitatively measure the effect of irradiation at low doses. These studies are being continued.
Studies on the analysis of survival curves for different radiations have been continued with the aim to evaluate whether differences in the LET dependence can be established between single track damage and damage due to accumulation of lesions.
From an analysis of data on the relative biological effectiveness (RBE) of many types of radiation, including heavy particles and neutrons, the suggestion has been derived earlier that for the type of damage causing the single track component, a composite interaction mechanism must be responsible, involving several primary changes. The steep increase of the cross-section versus LET curve between 20 and 100 keV/μm of tissue and the large RBE-values observed, indicate that for the induction of this damage several hundred eV of energy must be deposited in sites with dimensions of the order of 10 nm.
In order to explain what is implied by the suggestion of a composite interaction, it is of interest to hypothesize about the mechanism responsible for the single track type of damage. On the basis of many experimental results concerning the induction of DNA double strand breaks (DSB), it appears attractive to assume that these breaks are involved in the induction of reproductive death. A DSB is generally assumed to result from two single strand breaks (SSB) close together in the DNA, and therefore constitutes an effect which requires more than an amount of energy equivalent to a single ionization. i.e. more than about 30 eV. However, the most simple hypothesis that a single DSB is responsible for the induction of mammalian cell reproductive death appears to be incorrect. Firstly, a dose of 1 Gy of low- or high-LET radiation causes per cell 20-40 DSB's which evidently are not all lethal, because this dose will cause less than 50 per cent lethality in cells of average sensitivity. It must be concluded that most DSB's do not lead to cell reproductive death but are repaired.
In an analysis to be published separately (Barendsen, 1988), I have concluded that an alpha-particle with an LET of 100-200 keV/μm, which impinges on a spherical nucleus of a mammalian cell with 10 pg of DNA, will traverse 50-100 times through a DNA double strand of 2 nm diameter, 20-50 times through a nucleosome of 11 nm diameter or 3-10 times through a chromatin fiber of packed nucleosomes of 30 nm diameter. During such a traversal a large amount of energy is deposited, adequate to induce a composite type of damage. This is consistent with the observation that between 100 and 200 keV/μm the cross section for cell death is constant.

Based on the two types of conclusions from experimental data, i.e., the requirement of a large amount of energy deposited in a small volume with dimensions of the order of 10 nm and the suggestion that high-LET radiations produce unrepairable DNA lesions more effectively, it might be hypothesized that one single DSB is not sufficient for the induction of cell reproductive death but that two DSB's, if produced close together within a distance of the order of 10 nm, constitute the composite effective lesions responsible for cell reproductive death. This would imply that a structure with dimensions larger than the DNA helix of 2 nm diameter is the critical target. Candidates are the nucleosomes with a diameter of 11 nm and the chromatin fibers of packed nucleosomes, with a diameter of about 30 nm.
The hypothesis that lethal lesions induced by single tracks are the result of two DSB's produced sufficiently close together in chromatin fibres, may be extended to suggestions about potentially lethal lesions which might be associated with somewhat larger distances between two DSB's. The distance within which two DSB's have to be produced to cause cell death might be variable and part of the composite lesions might only be potentially lethal, depending on various factors, e.g., on the degree of super-coiling of the DNA and on the metabolic state of the cell. On the basis of this assumption it is possible to interpret differences in radiosensitivity among cells with respect to the initial slope of the survival curve. Only a small fraction of the total number of DSB's is known to be effective, i.e., the number of 20 to 40 DSB's produced by a dose of 1 Gy induces only an average of less than one lethal event. It is well established that almost all of the single strand DNA breaks and a large majority of the DSB's induced in mammalian cells by ionizing radiations are repaired. The composite interaction of two DSB's produced sufficiently close together in the critical structure might prevent their accurate repair and might subsequently result in loss of part of the DNA information. The distance between the two DSB's might be critical, however, with respect to probability that repair mechanisms can eliminate the lesions. Consequently, if this hypothesis is correct, it must be assumed that the initial slope of a survival curve depends strongly on the capacity of cells to repair DSB's.
The hypothesis of a composite DSB interaction would also be consistent with the observation that the RBE-values for cell lethality by single events are larger than RBE-values for DSB induction. It is evident that the probability of causing two DSB's close together by a single particle must depend more strongly on the LET than the induction of a single DSB.

IV. Objectives for the next reporting period:

Studies will be continued to obtain data and perform analysis of dose response relationships on the induction of chromosome damage and cell reproductive death of mammalian cells irradiated by different radiations. Measurements will be made to compare results derived by conventional methods and by flow karyometry.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Laboratory for Radiobiology, University of Amsterdam. Dr. J.A. Aten and Dr. J.B.A. Kipp.

VI. Publications:

- G.W. Barendsen: Chromosome abnormalities, transformation and reproductive death studied with different radiations and flow karyometry. In: Radiation Research,m Proceedings of the 8th Int. Congress of Radiation Research, Edinburgh, July 1987, Vol. 2, 568-574, 1987 (E.M. Fielden, J.F. Fowler, J.H. Hendry and D. Scott, eds.). Published by Taylor and Francis, London.
- J.A. Aten, M.W. Kooi, J. Stap, J.B.A. Kipp and G.W. Barendsen: X-ray and neutron-induced chromosome damage detected by flow cytometry compared to cell lethality and chromosome structural changes. Radiation Research, 110, 329-339, 1987.

Title of the project no.: 2

Transformation of cells in culture by ionizing radiations of different LET

Head(s) of project:

Prof.dr. G.W. Barendsen

Scientific staff:

G.W. Barendsen, H.B. Kal, and J.F. Gaiser

I. Objectives of the project:

The proposed research program will be carried out to obtain quantitative information and insights in the cellular processes occurring after irradiation which determine the development of malignant tumours. In particular, studies will be performed on the relative biological effectiveness of various types of radiations, which differ in the pattern of energy deposition in volumes of sub-cellular dimensions, e.g. chromosomes, because the results can provide information on the mechanisms by which cells are transformed, acquiring malignant characteristics, or are affected with respect to their proliferation and as a consequence have lost the capacity to express altered properties in their progeny. Differences between dose-effect relations obtained with radiations of different linear energy transfer can thus be used to test various hypotheses about carcinogenesis.
Studies on transformation of cells in culture are relevant to carcinogenesis because the clones with altered morphological characteristics can develop into tumours upon inoculation in syngeneic hosts.

II. Objectives for the reporting period:

For the induction of cell transformation it is evident that similarities in dose-effect relations and the dependence on radiation quality in comparison with the induction of chromosome aberrations and cell reproductive death are observed. But these cannot be considered as proof of a hypothesis that similar primary mechanisms are involved. Cell transformation, which is induced with a relatively low frequency compared to cell reproductive death or gross chromosome aberrations, could in principle be caused by a type of DNA damage which does not represent breakage of a DNA molecule. To obtain further insight, studies on chromosomal aberrations in clones of transformed cells will be continued.

III. Progress achieved:

The NBCH-3 diploid cell line has been employed to study cell transformation in vitro and to analyze chromosomal aberrations occurring in clones of transformed cells. Flow karyometry has been employed to study changes in chromosomes associated with transformation. Detectable alterations in flow karyograms imply that a change must have occurred in the primary transformed cell or in early generations of cells in a developing clone of these cells and that these changes have been transmitted to all or a large fraction of the cells in these clones.
The purpose of our studies was to analyse whether consistent changes can be detected, which are present in all clones of transformed cells and which can be assigned an essential role in transformation.
In these studies 48 clones have now been cultured but only 25 have been analysed in detail. A consistent pattern of chromosome aberrations could not be detected and it had to be concluded that changes occurring at different sites and on different chromosomes could be associated with the transformation mechanism.
On continued culturing of these clones of transformed cells more chromosome aberrations developed, indicating an inherent instability which in control cultures developed much later. It can be concluded that, although transformation is in an early stage associated with chromosome structural or numerical changes, there is not a specific site on only one chromosome which determines the transformation change. The hypothesis has been advanced that the earliest change in the genome induced by radiation which eventually causes the transformed phenotype, is a change which can be induced in several chromosomes. This change confers or enhances an instability in the DNA replication and, as a consequence, in subsequent cell generations specific cytogenetic changes may occur which eventually lead to carcinogenetic properties. The first change can occur at several sites on various chromosomes and can frequently be detected by karyometry. The subsequent changes involved in the development of carcinogenicity might be more specific. Thus cell transformation by radiation is a multistep process initiated by genomic destabilization. In this respect reproductive death and chromosomal aberrations microscopically observable at mitosis, are more directly caused by radiation induced damage to DNA than transformation.
Further studies have now been performed with "normal" clones of NBCH cells, which have developed from irradiated cultures, but which do not exhibit transformed characteristics. In some of these clones chromosome aberrations have also been observed, indicating that a singular correlation between chromosome aberrations and malignancy can not be deduced from these studies with cultured cells. Further studies on the pattern of chromosome aberrations in cultured cells will be performed to analyse whether a different pattern can be established for malignant and "normal" colonies.
Data on the influence of the dose rate of fast neutrons have been obtained for the NBCH-3 cell line. Dose-effect relationships for transformation frequency per surviving cells were measured for 1 MeV neutrons. It could be concluded that no enhancement of cell transformation is observed for a dose rate of 3 $mGy.min^{-1}$ compared to 150 $mGy.min^{-1}$ wiht frequencies of 0.8×10^{-3} and 1.5×10^{-3}, respectively. It should be noted that the uncertainties in these values are about a factor of 2 and the observed difference is not signifiant.

IV. Objectives for the next reporting period:

For the induction of cell transformation it is evident that similarities in dose-effect relations and the dependence of radiation quality in comparison with the induction of chromosome aberrations and cell reproductive death are observed. But these can not be considered as proof of a hypothesis that similar primary mechanisms are involved. To obtain further insight, studies on chromosomal aberrations in clones of transformed cells will be continued. Furthermore, studies on dose rate effects of neutrons of different energies for induction of cell transformation will be continued to investigate whether the enhancement of transformation described for C3H 10T½ cells can also be measured for NBCH-3 cells.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Laboratory for Radiobiology, University of Amsterdam, Dr. J.A. Aten and Dr. J.B. A. Kipp.

VI. Publications:

- G.W. Barendsen: Chromosome abnormalities, transformation and reproductive death studied with different radiations and flow karyometry. In Radiation Research, Proceedings of the 8th Int. Congress of Radiation Research, Edinburgh, July 1987, Vol. 2, 568-574, 1987 (E.M. Fielden, J.F. Fowler, J.H. Hendry and D. Scott, eds.). Published by Taylor and Francis, London.
- J.A. Aten, M.W. Kooi, J. Stap, J.B.A. Kipp and G.W. Barendsen: X-ray and neutron-induced chromosome damage detected by flow cytometry compared to cell lethality and chromosome structural changes. Radiation Research, 110, 329-339, 1987.

Title of the project no.: 3

Experimental studies on lung tumour induction by inhalation of radon in combination with some promoting agents, present in cigarette smoke.

Head(s) of project:

Prof.dr. G.W. Barendsen.

Scientific staff:

Prof.dr. G.W. Barendsen, Drs. Meijnders, Mr. J.S. Groen.

I. Objectives of the project:

The lung tumour risk for the general public, associated with the inhalation of radon daughters, is generally estimated on the basis of a risk factor, derived form epidemiological data on various exposed groups (e.g. uranium miners, A-bomb survivors and irradiated patients). The value of this risk factor is strongly determined by the data for miners, a group consisting of heavy smokers. From these data, conflicting conclusions, involving protective as well as synergistic action, have been inferred with respect to the risk of the combined exposure to radon and to tobacco smoke. Consequently, no definite risk for a non-smoking populatiron can be estimated.
It is the aim of this study to analyse such interaction mechanisms of combined exposure to radon with other inhaled toxic agents, in order ot improve insights in the values of estimates of the radon daughter risk.

II. Objectives for the reporting period:

For the study WAG/Rij rats will be epxosed to various regimes of radon, formaldehyde and acetaldehyde, respectively. As reported earlier, for the exposure of the rats ot radon daughters, a "nose-oniy" inhalation chamber combined with recycling and reconditioning of the air in a CO_2 absorber will be optimalied.
For the reported period, the plans for the construction and installation of this system were:
a. the assemblage of the CO_2 absorber;
b. measurements of the different radon daughter levels in the exposure system;
c. incorporation of the aldehyde generator in the system.

III. Progress achieved:

The radon chamber for exposure of rats has been completed and exposures of groups of rats to various radon concentrations have been started. Based on the newest data from the scientific research at Razes, France (Dr. Chameaud) it was concluded that the initially proposed exposures to 1000 and 2000 WLM would be too large to study synergism between Radon and acetaldehyde. Consequently, selected levels were 200 and 800 WLM, at which the expected lung cancer incidence would be about 10 and 30 per cent, respectively.
Exposures at these levels have started with 40 rats per group, either radon alone, acetaldehyde alone, and combined exposures to both agents, as well as appropriate sham treated controls. These exposures are still continuing, 50 per cent being completed by December 1988. Results on lung tumour development can be expected to be obtained in 1989 and 1990.

IV. Objectives for the next reporting period:

The plans for the coming year are:
a. measurements of the radon daughter concentration levels;
b. exposures of WAG/Rij rats to the two proposed radon daughter levels of 200 and 800 WLM.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

- Hogeweg, B. Eindrapportage en evaluatie van het SAWORA-onderzoeksprogramma naar het achtergrondniveau van de natuurlijke straling in Nederland. Rapportnr. 3477, Radiobiologisch Instituut TNO, Rijswijk (1986).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-070-I

Università degli Studi di Firenze
Piazza S. Marco, 4
I - 50121 Firenze

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Becciolini
Dipartimento di Fisiopatol. Clinica
Univ. degli Studi Policlin. Careggi
Viale Morgagni
I - 50134 Firenze

Telephone number: 55-434004

Title of the research contract:

Radiation carcinogenesis.

List of projects:

1. Follow-up of cancer patients treated by radiotherapy for the appearance of a second independent tumour.

Title of project no. BI6-D-070-I: RADIATION CARCINOGENESIS: FOLLOW UP OF CANCER PATIENTS TREATED BY RADIOTHERAPY FOR THE APPEARANCE OF A SECOND INDEPENDENT TUMOR

Heads of project: ALDO BECCIOLINI
ENRICO CELLAI

Scientific staff: DE MARIA D. (MO), FALCHI A.M. (MO), SANTONI R., BALZI M., CIONINI L., PORCIANI S., BOANINI P., MAURI P., SCUBLA E., ZANIERI E., LANINI A., BITI G.P., OLMI P., CHIAVACCI A., MUNGAI V., MUNGAI R., PACINI P., PUPI A., CINTOLESI V., FALLAI C., MAGRINI S.M., PONTICELLI P.

I. Objectives of the project: Carcinogenetic effect of ionizing radiations is well know but more data need to be collected to a better understanding of the phenomenon. Patients affected by cancer and treated by radiotherapy represent a very interesting object of study to evaluate the potential risk of induced cancer in humans exposed to radiations. Only in some Centers the clinical records of the treated cases are provided with enough data to assure a reliable evaluation of the incidence of second tumors after radiotherapy. Second tumors may appear in areas distant from the irradiated volume and the dose received by scattered radiation may be difficult to be defined. Also difficult is to determine the importance of the other factors involved in the etiogenesis of the primary tumor. Contemporary to the retrospective analysis of the clinical records, we also studied some biochemical indicators, tumor markers and cell kinetic parameters in new patients submitted to radiotherapy using conventional or multiple fractionation. The aim of this part of the study was to evaluate the acute and late radiation damage (including carcinogenesis) induced by different radiotherapy regimens.

II. Objectives for the reporting period: - Evaluation of the incidence of second tumors in patients treated at the Radiotherapy Institute, University of Modena.
- Analysis of the incidence of second tumors in patients previously treated with pelvic radiotherapy for gynecologic tumors at the Radiotherapy Sections of the University and of the U.S.L. 10/D of Florence.
- Continuation of the follow up of patients previously treated with different fractionation regimens and correlation with the appearance of second tumors.
- Study of the modifications of biochemical indicators, cell kinetic parameters and tumor marker in patients submitted to different fractionation regimens to evaluate the acute radiation damage.

III. Progress achieved.

A review of the clinical records of the patients seen at the Radiotherapy Section of the University of Modena between 1972 and 1987, was carried on to detect the incidence of multiple primary tumors. Out of 25000 patients overall scored presented 383 multiple primary tumors; in 86 they were sincronous and in 297 metacronous.
About a half of the second tumors were observed in patients with breast cancer, decreasing incidence was found in carcinoma of the cervix, head and neck tumors, colo-rectal tumors, endometrial carcinoma, ovary tumors, gastric tumors.
Out of the 217 patients treated by radiotherapy on their primary tumor 14 (6.45%) developed the second tumor in the irradiated volume. The time elapsed from the treatment to the appearance of the second tumor ranged between 8 and 28 years (mean = 17 years). All patients were irradiated with conventional fractionation (2 Gy x 1 time/day x 5 days/week up to a total dose of 45-55 Gy).
Six tumors of the rectum or endometry were observed in 2127 patients (0.28%) irradiated on the pelvis because of colon, bladder or uterine carcinoma. Three second tumors of the larynx and oral cavity (0.13%) were observed in 2225 patients previously treated with radiotherapy in the head and neck region. Three second tumors appeared in breast, larynx and lung in 1169 patients receiving thoracic radiotherapy because of malignant lymphomas (0.25%). Two cutaneous neoplasiae were observed in 2834 patients irradiated for breast cancer (0.07%). The mean incidence of second tumors appeared in irradiated volume was 1.67%.

From January 1977 to December 1985, 400 patients affected by cervical carcinomas, with advanced stages (clinical stages IIB - IV), were referred to the University and Hospital Departments of Radiotherapy of Florence. Patients were treated by a combination of radiotherapy and surgery (clinical stages II and III) or with exclusive radiation therapy (clinical stages III and IV). Patients with clinical stages IIB and tumors smaller than 4 centimeters in diameter received a single intracavitary insertion (50 Gy to point A) followed by radical surgery. Patients with positive lymph nodes were also submitted to pelvic irradiation.
In patients with an exophitic cervical tumor, but limited parametrial extension, unsuitable for intracavitary insertion, external irradiation was performed prior to radical surgery; postoperative radiation therapy was employed in all cases with positive lymph nodes. Patients with tumors larger than 4 centimetres, or with massive parametrial involvement, or classified as stage IV were submitted to exclusive radiation therapy.
A conventional fractionation regimen was used in all cases; the total dose delivered to whole pelvis, ranged between 50 and 70 Gy.
In this group of subject 12 presented a second neoplasm. In 5 the second tumor occurred before the diagnosis of cervical cancer; in 4 of them breast was the site of the second cancer and large bowel in the remaining one.

In two cases second tumor was syncronous; breast and endometrium were the respective site of origin.
In the remaining 5 patients a second tumor was found during the follow up after the cervical carcinoma. Breast cancer was the site in 3 cases while nasopharynx and larynx in the other two.

Biochemical indicators and tumor markers were assayed before and during radiotherapy in patients treated because of head and neck and gynecological cancer. In the first group of patients (H&N) the following fractionation regimens were used: 1) 2 Gy x 1 time/day x 5 days/week; 2) 1 Gy x 3 times/day x 5 days/week; 3) 2 Gy x 3 times/day x 5 days/week. Total dose was: 60 - 66 Gy in the regimens 1) and 2); 52 Gy in the regimen 3). In regimens 2) and 3) the interval between the fractions was 4 hours.
Overall 60 patients were studied. Serum TPA and α amylase proved to be valid indicators of acute radiation injury of salivary glands; an increase of both substances was in fact observed during the first days of radiotherapy.
TPA is also produced by neoplastic cells; TPA variations after irradiation may therefore also depend on tumor cells destruction induced by radiations. Pretreatment TPA values correlated with tumor size and tumor stage. Moreover, patients presenting a complete tumor regression (CR) after radiotherapy and maintaining such CR at 2 years had significantly lower TPA baseline values than patients presenting incomplete remission or recurrence (NR); the fraction of TPA-positive patients was also lower in the cases undergoing CR.
Only few patients had a follow up of 5 years: in this group only 17% had TPA baseline levels higher than the cut off value.
The post/pre-treatment ratio in patients in CR was close to 1.00 and no substantial modifications were observed. In NR patients, the ratio was significantly lower than in CR cases.
In the same patients urinary excretion of polyamine before and during radiotherapy was evaluated. Putrescine and spermidine concentration before treatment was related to the clinical stage. In T3-4N0 patients urinary putrescine and spermidine concentration was twice than in the T1N0 group.
Polyamine excretion varied during radiotherapy, however different patterns were observed in individual cases. Different patterns were also observed according to the used fractionation regimens and according to the treatment response. HPLC technique had recently evolved: the polyamine content of red blood cells can now be determined, and their behaviour can be directly studied before the renal catabolism.

IV. Objectives for the next reporting period: In the next future a new evaluation of clinical files during the follow up of irradiated patients will be done to record the appearance of new second tumors. Moreover the studies on biochemical indicators, tumor markers and cell kinetics parameters will be carried on to search a correlation between the damage in the irradiated organism and the progression of neoplasia or the promotion of a second tumor.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]: 1) Sezione di Radioterapia- Dipartimento di Fisiopatologia Clinica - Università Firenze
2) Sezione di Radioterapia - U.S.L. 10/D Firenze
3) Cattedra di Radioterapia - Università Modena
4) Sezione di Medicina Nucleare - Dipartimento di Fisiopatologia Clinica Università Firenze

VI. Publications: - A. Becciolini, M. Balzi, E. Scubla, P. Boanini
Modification of labelling index in tumors of the oropharynx during radiotherapy; XXXVI Ann. Meet. Radiat. Res. Soc., Book of Abstract p.93,1988.
- A. Becciolini, M. Balzi, S. Porciani
Il significato dei biomarcatori durante la radioterapia; in: Atti Simposio " I biomarcatori tumorali nella pratica clinica", a cura della XI U.S.L. Ge2, 235 - 256, 1988.
- M. Balzi, O. Gazzarrini, D. Cremonini, A. Sommavilla, E. Zanieri, F. Melone, A. Becciolini, M.C. Paoletti, A. Giannini, C. Biagini
The labelling index in tumors of human bladder; XXI Congr. Int. Soc. Urology, Buenos Aires, Book of Abstract, p. 26, 1988.
- L. Bandettini, M. Balzi, E. Scubla, D. Cremonini, A. Becciolini, A. Cardini
Cell kinetics parameters in the mucosa of patients affected by colonic tumors; XXVI World Cong. I.C.S., Milan, 1988.
- A. Becciolini, S. Porciani, E. Cellai, P. Olmi, M.S. Tommasi
Gli indicatori biochimici del danno da radiazioni; Aggiornamenti di Radiobiologia, D. De Maria, A.M. Falchi Eds.,ENEA 1988, in stampa

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Radiobiological Institute TNO
Division for Health Research
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

Contract no.: BI6-D-072-NL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. P.A.J. Bentvelzen
Division for Health Research
Radiobiological Institute TNO
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

Telephone number: 015-136940

Title of the research contract:

Molecular-biological studies on the activation of cellular transforming genes in radiation carcinogenesis.

List of projects:

1. Role of oncogenes in malignant transformation of mouse cells.

Title of the project no.:

Role of oncogenes in malignant transplantation of mouse cells.

Head(s) of project: P.A.J. Bentvelzen

Scientific staff:

V. Krump-Konvalinkova, P. van Klaveren, J. Dijk, A.G.M. Haaksma

I. Objectives of the project:

To test the hypothesis that radiation-carcinogenesis involves the separation of a proto-oncogene from a neighbouring cis-acting negative control element and subsequent translocation into the vicinity of a strong positive control element.

II. Objectives for the reporting period:

To characterise further an oncogene rescued from the tumour cell line T-neo-1, which has arisen after exposure of the murine fibroblast line NIH/3T3 to irradiate mouse DNA and plasmids containing the selectable marker neo^R, confirming resistance to the neomycin analogue G418.

III. Progress achieved:

A plasmid rescued from the T-neo-1 tumour line showed strong oncogenic activity in an assay in which transfected NIH/3T3 cells were inoculated soon after transfection into athymic nude mice. The rescued mouse DNA oncogenic sequence, called *tno*, gave strong bands in Southern blot hybridization with NIH/3T3 cells and additional faint bands with DNA rooms in the T-neo-1 line. In the Progress Report 1987 it was suggested that the faitness of the extra bands would reflect the polyclonality of the T-neo-1 line. However, all 6 different subclones derived from this line revealed the same hybridization pattern. In view of the aneuploidy of NIH/3T3 it may be assumed that the endogenous bands represent 3-4 copies of the *tno* gene per cell and the faint additional bands a single one.

The hybridization patterns of *tno* with DNA samples from different mouse strains and several other vertebrate species such as mice, rats, chicken and man ressembled that of the long terminal repeat (LTR) of the mouse mammary tumour virus (MMTV). Northern blot analysis of T-neo-1 RNA with this retroviral LTR as a probe revealed discrete MMTV-RNA species to be present in this cell line and absent from NIH/3T3 cells. The plasmid containing the *tno* sequence hybridized strongly with the MMTV-LTR but not with the *gag*, *pol* and *env* genes of this virus. It was assumed that the open reading frame contained in the MMTV-LTR would be the *tno* oncogene.

In six other NIH/3T3 sublines transformed by EcoR1-digested BALB/c mouse DNA, obtained in different experiments, additional bands were found with the MMTV-LTR as a probe in Southern blot analysis. This indicates that also in these experiments an endogenous MMTV-LTR sequence may have served as a donor oncogene.

In order to make sure that the MMTV-LTR sequence with its open reading frame would be the relevant oncogene, expression vectors with only the MMTV-LTR sequence were transfected to NIH/3T3 cells, which subsequently were inoculated into nude mice (10^6 cells per mouse). All mice got tumours within 4 weeks, whereas in control experiments no tumours developed within an observation period of 13 weeks. All tumours contained additional MMTV-LTR bands and expressed MMTV-LTR-RNA. These results strongly suggest that indeed the *tno* oncogene is identical with the MMTV-LTR.

The open reading frame of the MMTV-LTR was tested for the transactivating ability in a transient expression system. Expression vectors containing this open reading frame when co-transfected into NIH/3T3 cells with a vector, containing the prokaryotic chloramphenolacetyl-transferase (CAT) gene under control of the MMTV promoter,strongly enhanced CAT activity. A small deletion in the open reading frame of the LTR abrogated this enhancement; the CAT activity was as high as in the case of cotransfection with an irrelevant sequence. Similar enhancement was noted when the CAT gene under control of the MMTV promoter was transfected into rat mammary tumour cells infected with MMTV in comparison to uninfected cells. These experiments indicate that the open reading frame in the MMTV-LTR produces a protein, which can activate *in trans* the MMTV promoter. It is assumed that this viral gene also can activate in trans cellular genes, as also has been reported for the transactivating gene of the human T cell leukaemia virus. Most likely, the oncogenic action of the *tno* oncogene (identical with an endogenous MMTV-LTR) is due to such transactivating capacity.

In tumours, induced by the rescued plasmid with the *tno* oncogene, or by expression vectors with the open reading frame of the MMTV-LTR, as well as in the T-neo-1 line and NIH/3T3 clones transformed by EcoR1 digested BALB/c mouse DNA, a remarkable shift in the Southern hybridization pattern was noted. Using the restriction enzyme Bam HI and MMTV-LTR as a probe a thin but distinct endogenous 6 kb band had disappeared from all these tumours or transformed lines. Instead new bands of lower molecular weight (e.g., 4 kb) were found. These results are tentatively interpreted by homologous recombination: an MMTV-LTR sequence (*tno*) in the BALB/c mouse DNA samples or the exogenous viral transactivating gene in expression vectors would pair with an endogenous MMTV-LTR of NIH/3T3 cells and then become integrated by means of homologous recombination.

IV. Objectives for the next reporting period:

From the rescued plasmids the nonviral sequences, adjacent to the MMTV-LTR-homologous sequences, will be subcloned. These fragments will be used for Southern blot hybridization on NIH/3T3 cells and BALB/c mouse DNA as well as DNA from various NIH/3T3 sublines transformed by BALB/c mouse DNA. This will be done to ensure that the additional MMTV-LTR-sequences in the transformed cells are indeed derived from BALB/c mouse DNA.

By using dominant selectable markers the possibility of homologous recombination underlying neoplastic transformation will be studied in more detail. If this holds true it will be attempted to study this also in radiation-transformed NIH/3T3 cells.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

-

VI. Publications:

P. van Klaveren and P. Bentvelzen. Transactivating potential of the 3' open reading frame of murine mammary tumor virus. J. Virol. 62 (1980) 4410-4413.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-D-075-NL

Radiobiological Institute TNO
Division for Health Research
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.J. Broerse
Division for Health Research
Radiobiological Institute TNO
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

Telephone number: 015-136940

Title of the research contract:

Late effects in rhesus monkeys after whole body irradiation with X-rays and fission neutrons.

List of projects:

1. Incidence of cancer and non-stochastic diseases in an irradiated population of rhesus monkeys.

Title of the project no.:

Incidence of cancer and non-stochastic diseases in an irradiated population of rhesus monkeys.

Head(s) of project: Prof.dr. J.J. Broerse

Scientific staff: Prof.dr. J.J. Broerse and Dr. C. Zurcher

I. Objectives of the project:

Specific information related to the risk of radiation induced tumours or other late effects in man is limited. Data obtained from studies with larger animals and especially subhuman primates may be extremely valuable to assess the risk in man and also to estimate the RBE for tumour induction by neutron irradiation of human patients. In addition, the induction of non-stochastic effects in various tissues and the RBE of neutrons for these effects are of increasing importance for radiation protection problems. The present study on longevity, tumour induction and other late effects of total body irradiation of rhesus monkeys with fission neutrons and X rays has been in progress for about 20 years.

II. Objectives for the reporting period:

At this moment at about 3/4 of the estimated duration of the study, approximately 90 per cent of the irradiated monkeys have died compared with 40 per cent of the control group. All remaining monkeys receive a physical examination each month by a veterinarian with extensive experience with non-human primates for the clinical presence of tumours, cataract formation and changes in general condition. During the contract year two-X-irradiated animals died with malignant lesions. In the present communication the necropsy results of these two monkeys are reported.

III. Progress achieved:

The two groups of long-term surviving irradiated monkeys are part of a study on the effectiveness of bone marrow transplantation to prevent death due to the haemopoietic syndrome (Broerse et al., 1978). One group of long-term survivors consisted of nine macaques irradiated with fission neutrons with doses ranging from 2.3 to 4.4 Gy and the other of 20 X-irradiated monkeys which received doses between 3.0 and 8.6 Gy. A third group of 21 untreated rhesus monkeys of comparable age distribution was maintained under identical husbandry conditions to serve as a control group.
Within the contract year two X-irradiated rhesus monkeys died.
The male rhesus monkey 2652 (histology no. 1386/88 TBI X-irradiated 3 January 1974) died unexpectedly at 24 July 1988 after a few days of clinical illness at the age of 17 years. His body weight had decreased during the last year from 4500 to 3500 g. At necropsy and histological examination it appeared that he died from severe acute bronchopneumonia, secondary to severe pneumonyssus infection of the bronchial tree with fibrosis, bronchiectasis and emphysema. Septic abcesses with cocci were observed in the liver. In the right and left kidney papillary cortical carcinomas were observed (with diameters of 1.5 cm and 0.4 cm). Additional non-neoplastic findings were: right sided cataract, severe testicular atrophy, mild glossitis and oesophagitis due to candida infection; severe nodular hyperplasia and fibrosis in the right lobe of the liver; localized venectasies in the meninges with recent and organised thrombi, moderate to severe renal interstitial fibrosis and multiple smaller and larger cysts at the cortico medullary junction.
The female Rhesus monkey 2498 (histology no. 1946/88) treated with TBI X-rays on 27 November 1972, was euthanized December 12, 1988 at the age of 18 years, because of extreme emaciation (2100 g) and decline of general condition. At gross morphology only minor lesions, apart from the general wasting, were observed. Microscopically a small papillary cortical carcinoma was observed in the left kidney and a tubular adenoma in the right kidney. Uterine abnormalities were: multiple small leiomyomas, an endometrial polyp and multiple foci of endometriosis at the serosa. The most important non-neoplastic lesions were: severe septal fibrosis of the heart, severe fibrosis and atrophy of the exocrine pancreas with vacuolar degeneration of islet cells and moderately severe chronic superficial gastritis. These lesions could explain the clinically severe wasting disease.
Additional non-neoplastic findings were: chronic liver-congestion and liver cell atrophy, glossitis and oesophagitis due to candida, severe lymphoid tissue atrophy, renal cortical cysts and moderate interstitial fibrosis.

IV. Objectives for the next reporting period:

The group of animals which are still under observation comprises 12 untreated control monkeys, 1 neutron-irradiated monkey and 3 X-irradiated Rhesus monkeys. Every 6 months blood samples are collected for a complete haematological examination and serum samples are examined for the presence of paraproteinemia. The latter serological studies seem to be very promising as homogeneous immunoglobulins appeared to be twice as frequent in the irradiated groups as compared with the controls. Complete necropsies will be performed as soon as possible after natural death or euthanasia.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

This study is jointly performed by the Radiobiological Institute TNO and the Institute for Experimental Gerontology TNO. For the dosimetric aspects of the irradiations and the pathology, European collaboration has been established within the framework of the European Late Effect Project Groups (EULEP committees for dosimetry and pathology).

VI. Publications:

- Broerse, J.J., Van Bekkum, D.W., Hollander, C.F., and Davids, J.A.G. Mortality of monkeys after exposure to fission neutrons and the effect of autologous bone marrow transplantation. Int. J. Radiat. Biol. 34, 253-264, 1978.
- Broerse, J.J. Tumour induction in monkeys and rats after X- and neutron-irradiation. In Proc. Int. Conf. on Biological Effects of Large Dose Ionizing Radiation. Chinese Medical Association 319-337, 1988.
- Broerse, J.J., Van Bekkum, D.W. and Zurcher, C. Radiation carcinogenesis in experimental animals. Experientia, 45, 60-68, 1989.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: B16-D-219-NL

Academisch Ziekenhuis Leiden
Rijnsburgerweg, 10
NL - 2333 AA Leiden

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.J. Broerse
Afd. Klinische Oncologie K 1-48
Academisch Ziekenhuis Leiden
Rijnsburgerweg, 10
NL - 2333 AA Leiden

Telephone number: 071-261990

Title of the research contract:

Analysis of dose-effect relations for radiation carcinogenesis by various mathematical models.

List of projects:

1. Mathematical-statistical evaluation of data from animal and human studies and further development of statistical methods.

Title of the project no.:

Mathematical-statistical evaluation of radiation carcinogenesis data derived from animal and human studies.

Head(s) of project:

Prof.dr. J.J. Broerse

Scientific staff:

Prof.dr. J.J. Broerse, Dr.ir. J. Davelaar and Ing. J. Weeda.

I. Objectives of the project:

Quantitative estimates of the risk for breast cancer induction are essential for risk-benefit-cost analysis of mammography procedures. Large scale programmes on radiation-induced mammary neoplasms have been performed at relatively few laboratories in Europe and the United States. The results obtained at these institutes are analyzed by mutually different statistical models and associated computer programmes. A collaboration has been established between the institutes in the Netherlands and in the Federal Republic of Germany, to look for the implications of the different approaches. Special emphasis will be placed on the extrapolation of the animal data, to radiogenic risks of ionizing radiation for the human situation.

II. Objectives for the reporting period:

Experimental results on induction of mammary tumours in the rat for different exposure conditions (single and fractionated irradiation, sometimes combined with hormone administration) were available from earlier experiments. Different methods and models were applied on the same sets of experimental results from these large scale animal programmes with the aim of assessing the dependence on dose and exposure conditions with radiation carcinogenesis as the biological endpoint. The statistical methods applied at Neuherberg (Dr. D. Chemelevsky), the Department of Clinical Oncology and the Radiobiological Institute TNO have been investigated during working sessions at the three institutes.

III. Progress achieved:

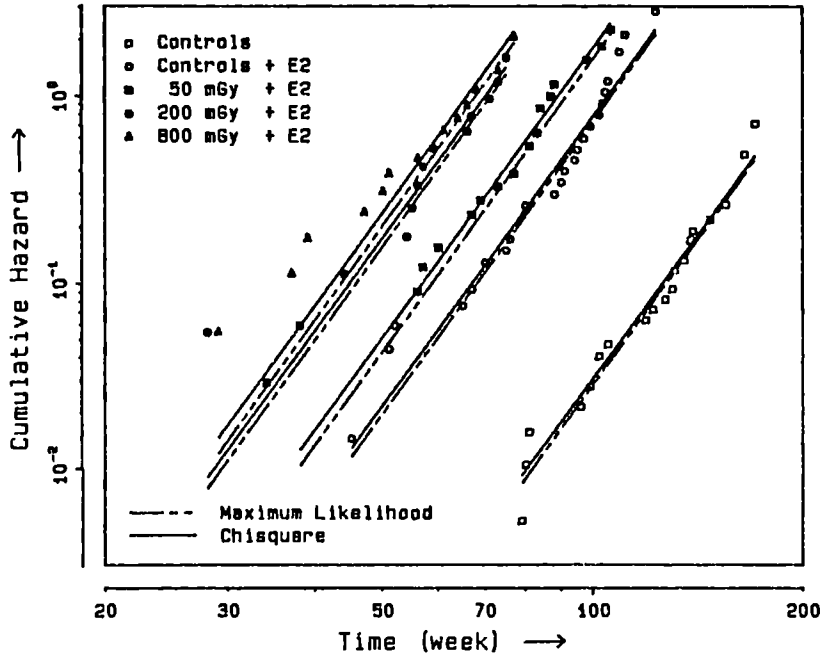
Ionizing radiation has been known to be an important agent for the induction of cancer. Both accidental and medical applications of radiation warrant the investigation of this carcinogenic effect with an emphasis on establishing the dose dependence. The sparse human epidemiological data has been supplemented for over a decade with animal experiments at the Radiobiological Institute TNO. Tumour induction was monitored at the mammary glands of various cohorts of rat strains, subjected to different doses and radiation quality.

Over the lifespan of the animals the possibly observed tumour induction has to compete with intercurrent deaths of animals due to unrelated causes, leading to so-called right censored data. Actuarial analysis with the Kaplan and Meier product or sum limit estimate will correct for these intercurrent deaths. The observed times for a tumour to be become palpable, generally denoted as failure times, are a measure of the hazard for the animals in the cohort. Microtumours observed upon obduction are assumed to represent failures at a time of 10 weeks thereafter (Broerse et al., 1986). The resultant survival curve for animals without evidence of tumour in the cohort are to be parametrized and quantitatively compared for the various cohorts in order to establish the dose dependence of the carcinogenic effect. One commonly assumed parametric function under these boundary conditions is a Weibull function of the survival S(t) with time (Broerse et al., 1985):

$$S(t) = \exp[-((t-\gamma)/\alpha)^{\beta}],$$

where γ is the time offset, α is the time scale parameter and β is the shape parameter. The optimization of this Weibull function to the data can be achieved through a χ^2 fit (Broerse et al. 1985) or the maximum likelihood method (Kellerer and Chemelevsky 1982). A good assessment of the validity of the assumption, that the survival curve is represented by a Weibull function is a log-log plot of the cumulative hazard, i.e. $-\ln(S(t))$ versus t, which should follow a straight line. Figure 1 shows this plot for a representative TNO data set of WAG/Rij cohorts, treated with X-rays and E2 hormone. A quantitative comparison of the methods of χ^2 and maximum likelihood analysis for the same dataset is presented in table 1. The time offset γ is fixed to zero, whereas the shape parameter β is common to the fit and the time scale α is variable. It should be noted that the TNO χ^2 method was newly adapted by us to an MS-DOS micro-computer and incorporated into a general analysis program written in PASCAL. The maximum likelihood analysis, adopted from the Würzburg/Neuherberg group, is also available in this program.

Although it is apparent from figure 1 that the Weibull function describes the cumulative hazard reasonably well, alternative models are also under consideration. Kellerer and Chmelevsky (1982) propose the time shift model as well as the proportional hazards model, which were recently discussed with one of authors at Neuherberg. The latter model is particularly attractive since no assumption on the shape of S(t) needs to be made. The application of the various models is also aimed at the experiments with protracted and fractionated radiation. The theoretical framework has been developed to incorporate these analyses in the PASCAL computer program.



Figuur 1. The cumulative hazard as a function of time in a log-log frame for both the data and the derived Weibull functions with either the maximum likelihood and χ^2 method.

Table 1. The derived parameters for the Weibull functions as in figure 1 with a common shape factor β and individual time scale factors $\alpha 1$ to $\alpha 5$ for the five cohorts.

		$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$
χ^2 (RBI/TNO)	β=5.13	189.0[1]	105.0	88.3	70.2	64.8
	Std. error= .18	3.8	1.0	1.1	1.2	1.1
χ^2 (Leiden)	β=5.13	197.5	105.1	89.2	70.2	66.1
	Std. error= .24	4.7	1.3	1.5	1.7	1.5
Maximum Likelihood	β=5.19	198.8	106.4	91.7	71.4	68.2
(Würzburg/Neuherberg)		9.3	3.8	3.8	3.8	3.5

[1] value is lower, since this control group was chosen slightly different.

IV. Objectives for the next reporting period:

The analysis of the protracted and fractionated experiments will be undertaken with the analysis software program developed at Leiden. For protraction an integral over the applied Weibull function will have to be implemented, whereas for fractionated experiments this will lead to the sum of Weibull functions. Alternative non-parametric analysis methods such as the proportional hazards model are also considered.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Institut für Medizinische Strahlenkunde, University of Würzburg, Federal Republic of Germany (A.M. Kellerer).
- Institut für Strahlenschutz GSF, Neuherberg, Federal Republic of Germany (D. Chmelevsky and H.G. Paretzke).
- Radiobiological Institute TNO and Institute for Experimental Gerontology TNO (D.W. van Bekkum, J. Zoetelief and C. Zurcher).

VI. Publications:

Broerse, J.J., Hennen, L.A. and Van Zwieten, M.J.: Radiation carcinogenesis in experimental animals and its implications for radiation protection, Int. J. Radiat. Biol. 48, 167, 1985.

Broerse, J.J., Hennen, L.A. and Solleveld, H.A.: Actuarial analysis of the hazard for mammary carcinogenesis in different rat strains after X- and neutron-irradiation, Leukemia Research, 10, 749, 1986.

Kellerer, A.M. and Chmelevsky, D.: Analysis of tumour rates and incidences, a survey of concepts and methods. In: Neutron Carcinogenesis, EUR 8084 (J.J. Broerse and G.B. Gerber, eds.), 209, 1982.

Broerse, J.J., Van Bekkum, D.W. and Zurcher, C.: Radiation carcinogenesis in experimental animals, Experientia 45, 60, 1989.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-088-F

Commissariat à l'Energie
Atomique, CEA
IPSN
B.P. n° 510
F - 75752 Paris Cédex 15

Head(s) of research team(s) [name(s) and address(es)]:

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Service d'Hygiène Industrielle
CEA/IPSN
B.P. n° 38
F - 26701 Pierrelatte Cédex

Telephone number: 75-50.43.80

Title of the research contract:

Studies into the actual toxicity of uranium compounds under conditions prevailing in the industry with a view to re-examining ICRP norms.

List of projects:

1. Studies into the actual toxicity of uranium compounds under conditions prevailing in the industry with a view to re-examining ICRP norms.

Title of the project no.:

Etude de la toxicité réelle des composés de l'uranium en situation industrielle

Head(s) of project:

Dr. J. Chalabreysse

Scientific staff:
E. Ansoborlo, M. Archimbaud, P. Bérard, M.H. Henge-Napoli

I. Objectives of the project:
Evaluer les risques réels, les caractéristiques physico-chimiques et les comportements métaboliques des composés d'uranium produits ou traités dans l'industrie nucléaire.

II. Objectives for the reporting period:
Poursuite des essais "in vitro" et "in vivo" afin d'étalonner les systèmes "in vitro".
Sur l'homme, essai de corrélation entre les études de poste et les résultats de radiotoxicologie

III. Progress achieved:

III.1 - ETUDE IN VITRO

Ces études de solubilité in vitro ont pour but de déterminer l'appartenance des composés industriels d'uranium aux classes D, W, Y de la CIPR.

Méthodology

Nous avons poursuivi l'étude des liquides de solubilisation afin de se rappprocher le plus possible des phénomènes observés in vivo (animal, homme) en utilisant un test statique défini dans nos études précédentes.

Des éléments ou composés nouveaux ont été testés :

- Introduction de l'oxygène et de l'anhydride carbonique par barbotage dans le liquide de Gamble (reproduction du phénomène respiratoire au niveau pulmonaire).

- Addition d'eau oxygénée (élément synthétisé au niveau du macrophage).

Les cinétiques observées sont exprimées sous forme d'exponentielles permettant de calculer la classe d'appartenance D, W ou Y d'un composé.

Results

Les résultats obtenus sur UF_4 sont très intéressants dans leur évolution :

- entre 1980 et 1983, une longue étude développée in vitro dans le liquide de Gamble a mis en évidence le caractère Y de UF_4 avec transformation chimique du composé,

- entre 1983 et 1987, nous avons comparé deux types de tests in vitro (dynamique et statique) et testé l'influence de certains composés (carbonates, phosphates). Nous avons constaté qu'en milieu carbonaté l'UF_4 prenait un caractère W alors qu'en milieu phosphate (inhibiteur), il était Y comme dans le liquide de Gamble,

- enfin entre 1987 et 1988, les essais en milieu "Gamble oxygéné" ont montré qu'UF_4 avait une tendance D, W, alors que l'ajout d'anhydride carbonique n'avait pas d'influence. L'addition d'eau oxygénée à Gamble (élément pouvant être synthétisé au niveau du macrophage) a montré une transformation chimique de UF_4 en UO_4, $3H_2O$, avec une solubilité faible de classe Y (fig. I).

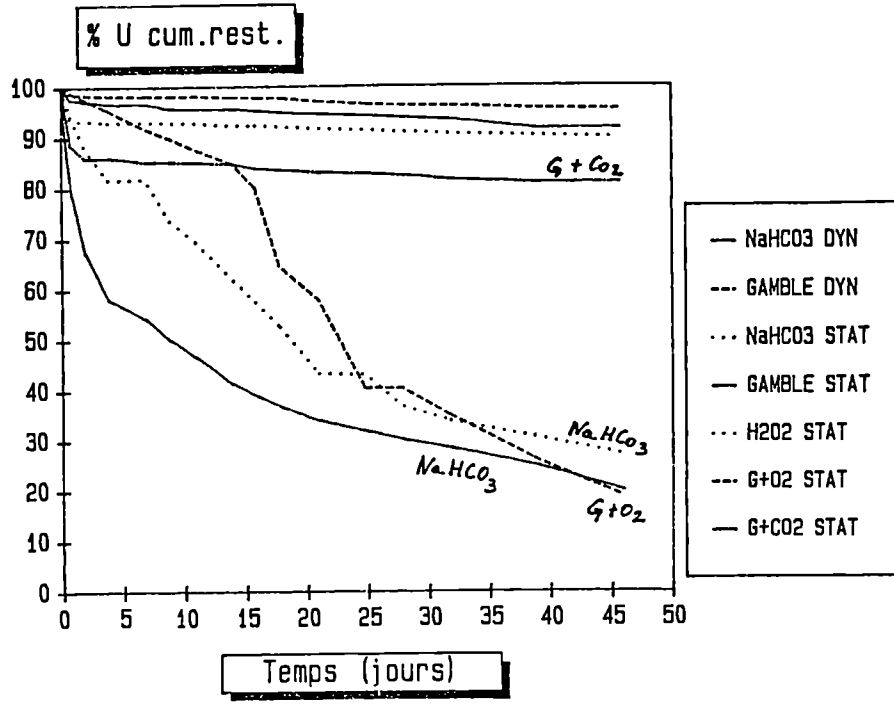


FIGURE N° I
Test de solubilité in vitro sur UF4 en dynamique et statique - Etude des carbonates, de Gamble (G) et de l'influence de l'oxygène et de l'eau oxygénée.

Le milieu Gamble + oxygène semble donc donner les mêmes résultats que pour les carbonates seuls et la classification D, W obtenue se rapproche des essais in vivo sur rats menés en parallèle.

Discussion

Cette évolution des tests in vitro sur 8 ans est très intéressante ; elle permet en outre de se rapprocher de plus en plus du modèle animal et humain.

En effet, l'UF4 est un cas exemplaire, car l'expérience humaine, acquise lors de la surveillance du personnel à Comurhex Malvési, a toujours montré, à travers les excrétions urinaires, qu'on était en présence d'un composé de type D.

Nous poursuivons actuellement ce développement de tests in vitro, en parallèle avec des tests in vivo chez le rat, en nous tournant vers l'étude de certaines protéines et la formation de radicaux libres.

III.2 - ETUDES IN VIVO

Etude in vivo de la toxicité de l'uranium et comparaison des cinétiques de solubilisation in vivo et in vitro.

Methodology

Expérimentation sur rats (OFA). Génération de poussières sèches. Le diamètre aérodynamique moyen, mesuré à l'aide d'un impacteur en cascade Andersen est de 6,6 ± 1,7 µm. La charge pulmonaire a été estimée en sacrifiant des animaux immédiatement après l'inhalation.

L'excrétion urinaire d'uranium a été suivie pendant 20 jours après l'exposition.

Results

La charge pulmonaire mesurée après inhalation d'UF4 était de 98 µg/rat. L'excrétion urinaire moyenne observée a été comparée à la courbe théorique calculée suivant le modèle de la CIPR (figure 2). La demi-vie calculée est de 3 jours.

Pour le composé NUFCOR (mélange U3O8 - UO3), la valeur moyenne de la charge pulmonaire était de 45 µg/rat. L'excrétion urinaire est celle d'un composé D, avec une demi-vie de 2,9 jours (figure 3).

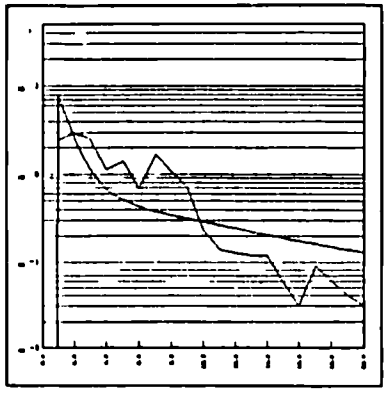


FIGURE II
1 - Excrétion urinaire moyenne des rats pour UF4
2 - Lissage suivant le modèle CIPR pour un composé D

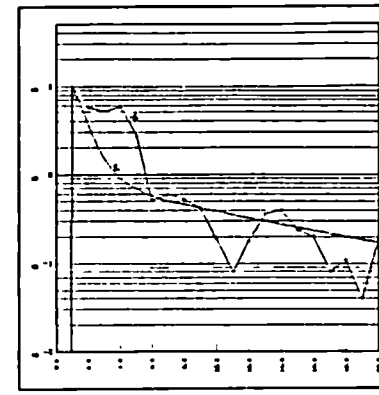


FIGURE III
1 - Excrétion urinaire moyenne des rats pour NUFCOR
2 - Lissage suivant le modèle CIPR pour un composé D

Discussion

Ce protocole devra être vérifié sur d'autres composés de l'uranium afin de le valider par rapport à des intoxications in vivo.

III.3 - ETUDES SUR L'HOMME

Valider les méthodes et les stratégies de surveillance radiotoxicologique des travailleurs exposés aux composés industriels d'uranium en France.

Methodology

Le Service d'Hygiène Industrielle, en liaison avec les Services de Médecine du Travail des exploitants nucléaires, assure la surveillance radiotoxicologique des travailleurs (urines, selles, anthropogammamétrie) ; en outre il effectue des études des postes de travail les plus représentatifs de l'exposition (concentration atmosphérique, granulométrie et solubilité biologique des poussières).

Il dispose ainsi des données suivantes =

- résultats radiotoxicologiques de personnels exposés,
- résultats des contrôles atmosphériques journaliers (APA),
- résultats des mesures des aérosols aux postes de travail.

Une recherche de corrélations est effectuée entre ces différentes données.

Results

A) Exposition chronique au tétrafluorure d'uranium (UF_4)

Les figures 1 et 2 représentent l'évolution des concentrations atmosphériques et urinaires de 1977 à 1988. Nous constatons la tendance très nette des 2 courbes à la diminution dans le temps (décroissance exponentielle), mais par contre pas de corrélation notoire entre ces 2 courbes, notamment au niveau des pics.
L'observation fréquente de 1977 à 1980 de pics urinaires importants suivis d'une décroissance rapide corrobore les résultats in vitro et in vivo classant l'UF_4 comme un élément transférable de classe D, W.

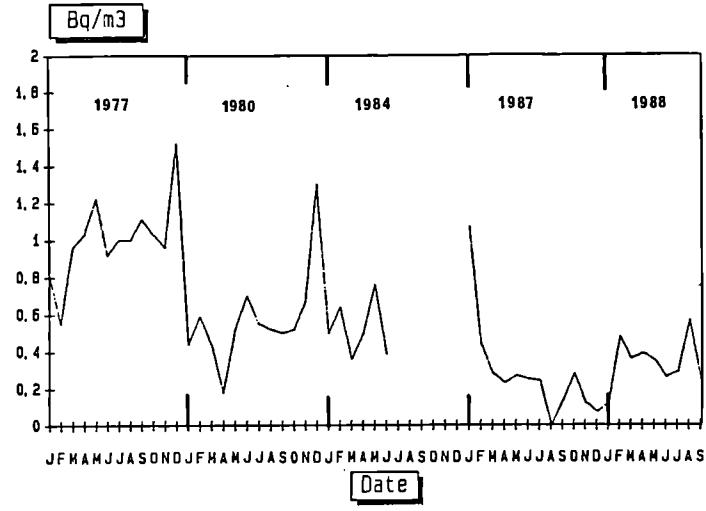


FIGURE N° 1
Evolution de la concentration atmosphérique en uranium de 1977 à 1988 dans l'atelier de fluoration

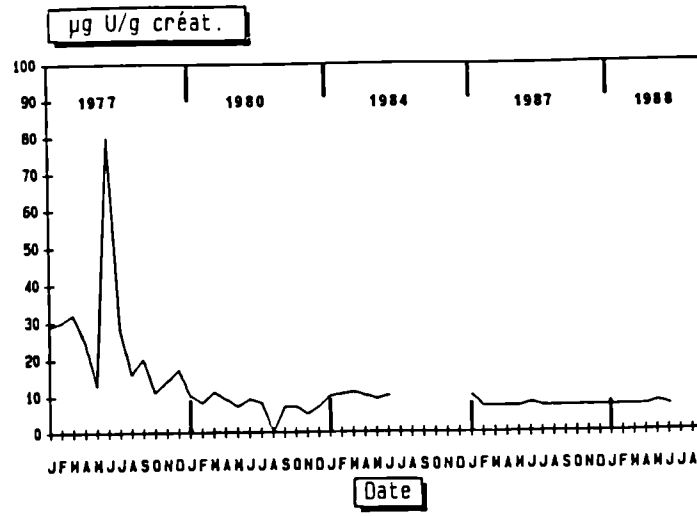


FIGURE N° 2
Evolution de la concentration en uranium de 1977 à 1988 dans le même atelier (urine).

B) Exposition aux oxydes d'uranium

La surveillance individuelle des travailleurs exposés aux oxydes d'uranium pose plusieurs problèmes :

- la détermination des populations à surveiller de manière permanente et celles pour lesquelles on ne fera des mesures que ponctuellement (sur incident ou pour des travaux particuliers ou pour sondage),

- la mise au point d'un protocole de surveillance fiable, permettant d'évaluer la dose interne, compatible avec l'organisation du travail.

L'expérience acquise montre que la réponse à ces questions nécessite une bonne collaboration entre :

- le médecin du travail,
- le responsable de la radioprotection, et son équipe,
- le laboratoire, les biologistes et spécialistes de radiotoxicologie.

En effet, pour l'évaluation de la dose interne, il est nécessaire de connaître :

- les résultats des mesures biométrologiques :
  . spectrométrie gamma du thorax,
  . excrétion fécale, complémentaire de celle-ci,
  . excrétion urinaire et prélèvements nasaux, utile en certaines circonstances.

- mais aussi les résultats d'examens portant sur le radio-contaminant lui-même et l'ambiance de travail.

A cet effet, ont été mises en place dans certains ateliers des opérations pilotes consistant en :

- mesures de granulométrie à l'impacteur Andersen, complétées par l'examen au microscope électronique,

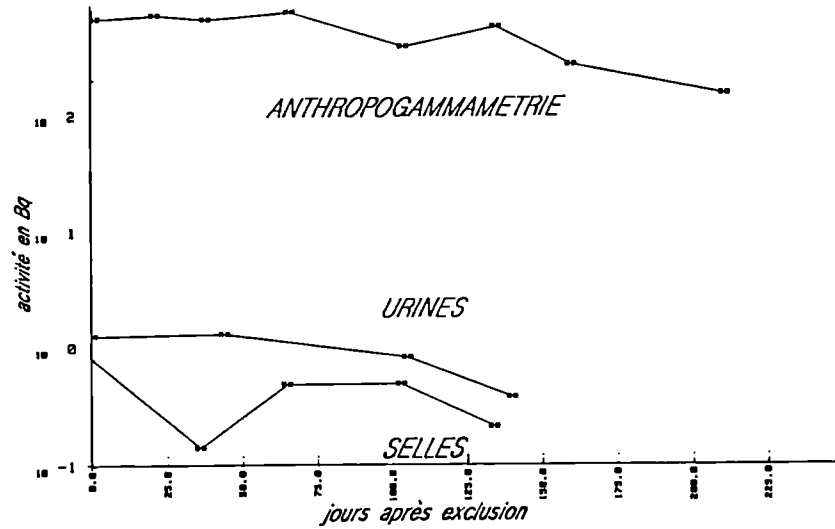
- mesure de la fraction inhalable par appareil de prélèvement individuel portatif,

- spectre de diffraction aux rayons X, mesures de densité et de surface spécifique.

L'ensemble de ces résultats, encore trop parcellaires pour conclure, paraît montrer une assez bonne concordance avec le modèle pulmonaire avec cependant une excrétion urinaire souvent plus imporante et une période biologique plus courte (cf. FIGURE N° 3).

FIGURE N° 3

EVOLUTION DES EXAMENS APRES EXCLUSION
Aérosols de classe Y : -- UO2 -- U3O8 --
Pastillage



Discussion

Les travailleurs ne sont en général pas exposés à leur poste de travail à des produits purs. En outre, les conditions réelles de fonctionnement des procédés industriels sont variables dans le temps. Ceci rend difficile la recherche de corrélations entre résultats radiotoxicologiques humains et les concentrations atmosphériques. Néanmoins, d'ores et déjà, la connaissance des excrétions urinaires et fécales couplées aux rétentions pulmonaires permet une bonne approche de la dose interne contribuant ainsi à la protection des agents.

IV. Objectives for the next reporting period:

Poursuite des essais in vivo et in vitro, en particulier sur UO3.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Docteur Beau - DPS/SEGP - CEA - FONTENAY AUX ROSES
- Société COMURHEX - DR. GIBERT - MALVESI FRANCE
- Société FBFC - DR. BOURDEIX - ROMANS FRANCE

VI. Publications:

- HENGE-NAPOLI M.H., RONGIER E., ANSOBORLO E., CHALABREYSSE J.
  "Comparison of the dissolution rates of diuranates obtained "in vitro" with those obtained "in vivo" in rats and research of an early urinary indicator of renal failure in human and animals poisoned with uranium". Workshop - Biological assessment of occupational exposure to actinides - VERSAILLES - 30 mai - 2 juin - 1988.

- CHALABREYSSE J., BEAU P.G., CHEVALIER C., JEANMAIRE L., BATALLER C., BERARD P., GIBERT B.
  "Expérience acquise en France sur les composés de l'Uranium"
  Workshop - Biological Assessment of occupational exposure to actinides - VERSAILLES - 30 mai - 2 juin - 1988.

- ANSOBORLO E., BERARD Ph., CHALABREYSSE J. -
  "Etude de l'exposition industrielle à des composés de l'uranium de classe Y : méthodes et résultats"
  Workshop - Biological assessment of occupational exposure to actinides - VERSAILLES - 30 mai - 2 juin - 1988.

- BEAU P.G., CHALABREYSSE J. -
  "Mise en évidence par le retour d'expérience, de particularités métaboliques et toxicologiques de l'UF6 et de ses produits de dégradation". Workshop - Biological Assessment of occupational exposure to actinides - Versailles - 30 mai - 2 juin - 1988.

- BOURDEIX F., ACHIARY J., BERARD Ph.-
"La surveillance de l'exposition interne aux oxydes d'uranium dans les usines de fabrication du combustible" - Communication aux Journées URANIUM les 10, 11, 12 et 13 octobre 1988 à Montpellier.

- CHALABREYSSE J. -
Les études menées en radiotoxicologie de l'uranium par le Groupe de Travail 8-1 du Conseiller Médical - Communication aux Journées Uranium les 10, 11, 12 et 13 octobre 1988 à Montpellier.

- ANSOBORLO E., GIBERT B., CHALABREYSSE J. -
Exposition chronique aux composés uranifères : problèmes médicaux de surveillance liés à leurs propriétés physico-chimiques et leur solubilité = données actuelles et perspectives" - Communication aux Journées URANIUM les 10, 11, 12 et 13 octobre 1988 à Montpellier.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-076-UK

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. L.M. Cobb
Radiobiology Unit
MRC
Harwell, Didcot
GB - Oxon OX11 ORD

Telephone number: 0235-834393

Title of the research contract:

Local retention and translocation of particles in the respiratory tract.

List of projects:

1. Mechanisms governing particle translocation and related aspects of lung function.
2. Spatial distribution of particles in the lung in relation to cells at risk.

Title of the project no.: 1

Mechanisms governing particle translocation and related aspects of lung function

Head(s) of project:

Dr. G. Patrick

Scientific staff:

Dr. G. Patrick

I. Objectives of the project:

The primary aim is to clarify those cellular and physiological processes which determine the movement of particles within the respiratory tract, especially to sites which are important in lung dosimetry. The approach is based mainly on methods for the selective deposition of particles in specific regions of the respiratory tract of experimental animals. Techniques developed for this purpose may also permit a related study of the permeability of the alveolar epithelium.

II. Objectives for the reporting period:

The main objective was to continue a study of the long-term kinetics of alveolar clearance of colloidal gold particles, following micro-injection into subpleural alveoli of rat lung. Lung tissue was to be obtained from the animals when sacrificed at different times after microinjection, which would be studied in turn by radioassay, autoradiography and electron microscopy. One purpose was to obtain information on the redistribution of particles within the respiratory tract, following initial deposition in a single very small volume of tissue.

III. Progress achieved:

Methodology

For the long-term study of alveolar clearance, 38 F-344 rats had been injected with a suspension of colloidal gold particles, as reported previously. The colloid was injected by a micro-pipette passed through the parietal pleura of the left lung. The gold was labeled with ^{195}Au; the amount injected was not greater than 1 µg Au in 0.3 µl suspension.

Eight of the rats were assayed repeatedly for ^{195}Au in the thorax, using a small animal whole-body counter. Other animals were serially sacrificed after intervals of 4 minutes, 1 day, 1 week, and 1, 4, and 9 months. The left lung was divided along its major axis into 2 mm slices. The slices containing most ^{195}Au were either sectioned for autoradiography or were cut into 2 mm cubes for further processing for electron microscopy.

Results and Discussion

All of the animals in the long term study have now been killed. Of the group studied by repeated thorax counting, the six rats surviving to 15 months after injection were killed at that time. Observations on this groups of rats had previously shown that there was no rapid clearance of particles from the subpleural site: the mean lung burden, as a percentage of the initial value, was 91 % at 1 week after injection and 86% afer 2 weeks, only falling to 70% 10 weeks after injection. The final lung burden of this same group, at 15 months after microinjection, was approximately 45% of the initial value. Interestingly, the range of values among the six surviving rats was quite large. Bearing in mind that the particles were injected into a very small volume of lung tissue, this variation between rats may reflect differences in the position of the deposition site in relation to the pattern of alveoli, alveolar ducts etc. in the immediate vicinity.

On dissection it was found that, of the ^{195}Au retained in the body, more than 90 % was retained in the left lung at all times after injection. By 9 months, the proportion retained in the thoracic lymph nodes had increased to 2.6 $\pm$ 0.4 %, as compared with 2.2 $\pm$ 1.8 % in the liver, 0.02 $\pm$ 0.015 % in the spleen and 0.50 $\pm$ 0.23 % in the kidneys.

Radioassay of slices of left lung has shown that the initial

deposit was confined to within 1-2 mm along the length of the lung. The pattern did not change appreciably over the whole period of the study. This demonstrates that there is no widespread redistribution of particles around the lung even over a long time period, such as might have been brought about by the migration of alveolar macrophages.

A large number of histological sections has been examined, cut from whole transverse sections of the left lung. These have been screened with X-ray film for the presence of ^{195}Au, and liquid emulsion autoradiographs have been prepared using selected sections. The study of these sections is still in progress but some interesting findings are already apparent. The sections from rats killed immediately after microinjection have confirmed that the area of deposition of gold particles extended into the left lung only approx 0.5-0.75 mm beneath the pulmonary pleura. Throughout the whole period up to 15 months by far the greater part of the gold retained in the lung remained in this region, seen on the autoradiographs as discrete foci which presumably represent alveolar macrophages.

From 1 day after injection right through to 15 months, there is evidence of gold particles, presumably mostly in macrophages, on the surface of the ciliated airways. At first these were seen mainly close to the deposition site, but from 1 week onwards they were also observed on larger airways right up to the hilum. The frequent discovery of macrophages containing the gold particles on ciliated airways long after microinjection provides important evidence that these cells, which originated in the subpleural alveoli where the gold was ingested, are not being rapidly cleared from the airway surface. How this can happen remains to be explored.

From 1 week onwards gold particles were also evident in the interstitium around the airways; from 1 month onwards they were seen around airway bifurcations including near the hilum. At 4 months after injection some particles were noted in lymphoid tissue near the hilum.

Many of these observations from the autoradiographs will require clarification by electron microscopic studies, which are still in progress.

IV. Objectives for the next reporting period:

The long-term study of gold particle clearance from rat lung alveoli, as described above, will be concluded. In particular, the autoradiographic study of gold particle distribution in the lung will be extended, and detailed observations made by electron microscopy to elucidate the cellular mechanisms of particle clearance. Additional supplementary experiments will be made to assist in the interpretation of particle clearance kinetics from the alveolar region.

If it proves technically feasible, a study will be made of the long-term retention of particles in association with the large airways of the rat when relatively insoluble particles are administered by inhalation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Patrick G. and Stirling C (1988) The clearance of particles of colloidal gold from subpleural alveoli. Annals. Occup. Hygiene 32, suppl. 1, 1164 1166.

Patrick G. (in press) Requirements for local dosimetry and risk evaluation in inhomogeneously irradiated lung, in Low Dose Radiation - Biological Bases of Risk Assessment, Proceedings of 14th L H Gray Conference, eds. K.F. Baverstock and J.W. Stather.

Title of the project no.: 2

Spatial distribution of particles in the lung in relation to cells at risk.

Head(s) of project:

Dr. G. Patrick

Scientific staff:

Dr. A.L. Batchelor
Dr. K.J. Morris

I. Objectives of the project:

The aim is to make a quantitative assessment of the spatial distribution of particles retained in the lung at long times after inhalation. Using human lung, measurements are to be made of the distances of occupationally inhaled particles from defined target cells, e.g. in the airway epithelium. This should permit an assessment to be made of the dose to the target cells in the lung from alpha-emitting particulates. Parallel studies are also to be made in the rat to study the spatial distribution of particles with respect to the airways up to two years after inhalation.

II. Objectives for the reporting period:

Human lung specimens from tin miners were to be analysed for spatial distribution of tourmaline-containing particles as they became available. The clearance rate of tourmaline from rat lung was to be finally evaluated and compared with corresponding clearance rates for inhaled insoluble actinide aerosols. Final lung clearance and gross body burden data were to be obtained in the long-term UO_2 inhalation study in the rat. The distribution of the inhaled UO_2 particles in the lungs was then to be examined. The ultrastructure of pulmonary cells was to be further studied in rats exposed to the same UO_2 aerosol. The method for the imaging of boronated tissue sections on CR-39 plastic was to be further refined.

III. Progress achieved:

Tourmaline-containing particle retention in human and rat lung

No new post-mortem human lung specimens became available. However, the techniques for analysing the tin miner lungs are fully developed and the work will continue as and when new Cornish tin miner and control lungs are obtained.

Measurements have continued and are now completed on the retention of tourmaline rock dust particles, labelled with ^{54}Mn, in rat lung. The pulmonary fractional clearance rate constant for the labelled particles was approximately constant at 1.5×10^{-2} d^{-1} between 3 and 90 days post-inhalation. An upper limit of 3.6×10^{-3} d^{-1} was estimated for the fractional dissolution rate constant of the ^{54}Mn label retained in the lung. No significant difference was found when the fractional pulmonary clearance rate for the tourmaline particles was compared with the rates for clearance over similar times of inhaled plutonium oxide or mixed plutonium and uranium oxides in rats. It was concluded that tourmaline is a suitable model for PuO_2 distribution in human lung.

Uranium dioxide retention in rat lung

The protocols for the long-term UO_2 inhalation studies have been described in previous reports (1986, 1987).

The full lung clearance data and related changes in body burden of inhaled UO_2 were obtained for all rats killed from 7 days to 720 days post-inhalation. All of the CR-39 neutron-induced autoradiographs required for the spatial distribution study have now been obtained, with the study including the rats sacrificed at 0, 7, 15, 30, 90 and 180 days post-inhalation in addition to those killed at 12, 18 and 24 months.

The original Quantimet 720 image analyser has been replaced with a Seescan Solitaire-plus grey scale analyser. This has been coupled to a high resolution camera system on a microscope fitted with a semi-automatic mechanical stage. In addition to this developmental work, new computer software has been written for the distribution study. This has now been completed and measurements with the CR-39 autoradiographs have started. The numbers of fission tracks are being counted in different

defined anatomical regions of lung tissue, e.g. associated with or near to airways.

The transmission electron microscope study of the effect of enriched UO_2 particles on cells in the alveolar region of rat lung has now been completed. Evidence was obtained that the inhalation of UO_2 particles significantly increased the size of macrophages and Type II epithelial cells, and the number of macrophages and Type I cells. The lysosomal granules within macrophages were increased in size. The toxicity of the UO_2 appeared to reduce the effectiveness of the alveolar macrophages to clear the UO_2 particles (Morris et al, in press).

The new tissue imaging technique using boronated tissue sections to produce CR-39 neutron-induced autoradiographs has been further refined (Morris and Batchelor, 1988). It is now possible to produce highly detailed tissue images on the CR-39 plastic, against which fission tracks from incorporated ^{235}U or ^{239}Pu can be readily distinguished. The position of the actinide can thereby be determined in relation to histological structures, without the need for a secondary shadow-imaging step using low energy α-particles.

IV. Objectives for the next reporting period:

The analysis of the spatial distribution of UO_2 particles in autoradiographs of rat lung will be continued. The human lung work will continue as and when new post-mortem tissue becomes available.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Harwell Laboratory (AERE), Oxon, U.K.

Department of Physics, University of Bristol, U.K.:
Dr. D.L. Henshaw

Atomic Weapons Establishment, Aldermaston, Berkshire, U.K.

VI. Publications:

Morris, K.J. and Batchelor, A.L. (1988) The simultaneous imaging of boronated tissue sections and the location of fissionable actinide particles in CR-39 solid state track detector, utilising a neutron-induced autoradiographic technique. Physics in Medicine and Biology, 33, 1195-1203.

Morris, K.J., Townsend, K.M.S. and Batchelor, A.L. (in press) Studies of alveolar cell morphometry and mass clearance in the rat lung following inhalation of an enriched uranium dioxide aerosol. Radiation and Environmental Biophysics.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-D-220-B

Université Libre de Bruxelles
av. F.D. Roosevelt, 50
B - 1050 Bruxelles

Head(s) of research team(s) [name(s) and address(es)]:

Prof. J.E. Dumont
Inst. Interdisciplinary Research
Université Libre de Bruxelles
Route de Lennik, 808
B - 1070 Bruxelles

Telephone number: 02-568.41.34

Title of the research contract:

Thyroid Radiation: Carcinogenesis in experimental models and effects of low doses in humans for risk assessment.

List of projects:

1. Studies on radiation risks to the thyroid in man exposed to X-rays or radioiodine and investigations on cell kinetics and transformation of isolated human thyroid cells.

Title of the project no.:

Thyroid Radiation: Carcinogenesis in experimental models and effects of low doses in humans for risk assessment.

1. Studies on radiation risks to the thyroid in man exposed to X-rays or radioiodine and investigations on cell kinetics and transformation of isolated human thyroid cells.

Head(s) of project:

DUMONT, J.E., M.D., Ph.D.

Scientific staff:

S. SWILLENS Ph.D.
P. ROGER Ph.D.
V. de MAERTELAER Ph.D.
J. VAN SANDE Ph.D.
P. GALAND Ph.D.
S. REUSE M.S.
M. TATON M.S.

I. Objectives of the project:

1. Development of a theoretical model of protein target inactivation analysis
2. Definition of cell proliferation mechanisms in the thyroid cell
3. Definition of oxygen detoxifying mechanisms in the thyroid and their role in carcinogenesis
4. Development of an experimental model of human thyroid cells in culture
5. Definition of cell kinetics of the human thyroid
6. Human thyroid tumors in vivo : definition of cell regulation alterations; search for oncogenes and gene rearrangements
7. Definition of the epidemiology of X ray induced human thyroid tumors

II. Objectives for the reporting period:

1. Development of a theoretical model of protein target inactivation analysis
2. Definition of cell proliferation mechanisms in the dog thyroid cell as a model
3. Demonstration of glutathione peroxidase and superoxide dismutase in the thyroid; effect of Selenium deficiency on thyroid physiology and radiation induced tumors
4. Development of an experimental model of human thyroid cells in culture
5. Definition of cell kinetics of the human thyroid
6. Definition of cell regulation alterations in autonomous nodules; search for transforming oncogenes and thyroglobulin rearrangements in human tumors
7. Definition of the epidemiology of X ray induced human thyroid tumors

III. Progress achieved:

1. DEVELOPMENT OF A THEORETICAL MODEL OF PROTEIN TARGET INACTIVATION ANALYSIS

Enzyme inactivation by irradiation has been widely used for determining the size and the molecular organization of enzymic systems in situ, because this method does not require the solubilization nor the purification of the enzyme.

On the basis of theoretical simulations, we have demonstrated the limitations of this methodology when applied to complex systems. We have shown that the sensitivity of the enzyme activity to irradiation not only depends on the size of the components of the system, but also on the relative concentrations of these components and on the kinetic parameters of the interactions between them. Therefore, in such systems, an apparent aberrant target size may lead to erroneous interpretations as, for instance, the existence of the aggregation of functional units. A more extensive study of equilibrium systems has led to the conclusion that interpretations based on target-size analysis are acceptable if and only if the complete description of the system is known concerning the components and their respective interactions.

A more fundamental work has been developed concerning the mode of action of the ionizing radiation on the biochemical function of the target. This theory based on a stochastic multievent process has been proposed in order to account for exerimental results, like the temperature effect, that were not explained by the classical single-hit model.

2. DEFINITION OF CELL PROLIFERATION MECHANISMS IN THE THYROID FOLLICULAR CELL

Methods

Culture of dog thyroid cells. Measurement of proliferation by cell counting, DNA measurement, ^{3}H thymidine incorporation into DNA and ^{3}H thymidine labeling of nuclei. Study of phosphorylated and induced proteins by 2.D 2 isotopes gel electrophoresis with ^{32}P phosphate and ^{35}Se methionine as tracers. Differentiation is evaluated by iodide transport and thyroglobulin gene expression (Tg mRNA by Northern blotting). Measurement of cMyc and cFos mRNA by Northern blotting.

Results

Thyrotropin, through cyclic AMP, enhances both proliferation and differentiation in the follicular cells. Epidermal growth factor (EGF) and phorbol esters enhance proliferation and inhibit differentiation. Fibroblast growth factor and serum enhance proliferation without interfering with differentiation. DNA synthesis starts 18 hours after the beginning of stimulation by any of these agents. The factors must be present continuously. The proteins phosphorylated after stimulation have been studied. EGF and phorbol esters induce the phosphorylation of a common set of proteins, while another set is only phosphorylated in the presence of phorbol esters. No common protein is phosphorylated in response to activation of the cyclic AMP cascade and TSH on the one hand and in response to the phosphatidylinositol Ca^{++} and the EGF protein tyrosine kinase cascades on the other hand.

With regard to protein synthesis the same pattern is observed. TSH induces one protein whose kinetics is compatible with a role in proliferation induction. EGF, serum and phorbol esters induce another such protein. TSH and EGF + serum potentiate each others actions on both proliferation and protein induction. cMyc and cFos mRNA concentrations are increased by all stimulants but with widely different kinetics. As in other cell types EGF and phorbol esters enhance the rapid transient accumulation of cFos mRNA followed by a longer accumulation of cMyc mRNA. On the other hand, TSH and activators of the cyclic AMP cascade enhance first and very shortly the accumulation of cMyc mRNA; the accumulation of cFos mRNA presents the same kinetics as after EGF and phorbol esters.

Discussion

Three different but partially overlapping pathways controlling cell proliferation have been delineated in the thyroid. The TSH cyclic AMP cascade, the Ca^{++} phosphatidylinositol cascade and the EGF protein tyrosine kinase cascade. While the first pathway enhances both proliferation and differentiation expression, the other two stimulate proliferation but inhibit differentiation. Biochemical steps of the three cascades have been demonstrated. While the cyclic AMP pathway is mostly distinct from the EGF and phorbol esters pathways, the biochemical steps of the latters are common after the initial interaction with the relevant receptors. The hypothesis has been proposed that activation of the cyclic AMP cascade leads to hyperfunction and proliferation eg. autonomous benign tumors, activation of the other cascades leads to non functional malignant tumors.

3. DEFINITION OF OXYGEN DETOXIFYING MECHANISMS IN THE THYROID AND THEIR ROLE IN CARCINOGENESIS

Methods

Measurements of superoxide dismutase and glutathione peroxidase in thyroid by classical methods. Induction of thyroid tumors in rats by administration of 30 uCi ^{131}I followed by appropriate diet; histological examination of the thyroids after 12 months.
Measurement of thyroid function in rats with selenium deficient combined with perchlorate supplemented diets. Pregnant rats were submitted to a selenium deficient diet immediately after mating; it was continued for 4 weeks after delivery. The pups were sacrificed at 3 and 4 weeks of age. Perchlorate, an antithyroid agent inhibiting iodide trapping in the thyroid, was administered via the drinking water to half of the rats. Rats submitted to a normal laboratory diet and to the experimental diet supplemented with selenium were used as controls.

Results

The effects of selenium deficiency were an increase in the number of growth abnormalities, growth retardation and decreased seleno-dependent glutathione peroxidase (GSH-Px) activity in plasma and in various organs. These effects were relieved by selenium supplementation in the diet. Perchlorate treatment induced the classic picture of primary hypothyroidism. Selenium deficiency increased thyroid hormone levels in perchlorate-treated rats and in controls drinking tap water. In the latter group, it also decreased TSH plasma concentration and thyroid weight. These effects were partially reversed by Se supplementation. In vitro experiments, performed on adult rats, revealed increased radioiodide

uptake and organification in glands from the rats submitted to the selenium-free diet. Plasma T3 half-life was similar in control and Se-deficient rats.

Selenium dietary supply has been investigated in human populations. While Belgians appear to have a normal supply, a marked deficiency is observed in Zaire. Other African countries are now investigated.

Administration of ^{131}I followed by iodine deficiency (as induced by a $NaClO_4$ supplementation in the water) causes after one year in 100% of the treated rats, thyroid cancers whether the rats are selenium deficient or not.

Discussion

Protection mechanisms against O_2 and its radicals have been demonstrated in the thyroid. Reduction of glutathione peroxidase levels in the thyroid by selenium deficiency alters the function of the rat thyroid. An important selenium deficiency in Africans may be responsible for the characteristics of endemic cretinism on this continent. However we have been unable to demonstrate an increased thyroid tumorigenic response to ^{131}I and iodine deficiency in selenium deficient rats.

4. DEVELOPMENT OF AN EXPERIMENTAL MODEL OF HUMAN THYROID CELLS IN CULTURE

Methods

Normal thyroid tissue obtained at emergency autopsies or from patients operated for simple nodules is treated with collagenase. Follicles are seeded and cultured as previously described in the presence or absence of 1% fetal calf serum. Differentiation is measured by iodide trapping, proliferation by counting ^{3}H thymidine labeled nuclei, or DNA measurement. Cyclic AMP and patterns of protein synthesis (^{35}Se methionine incorporation in the proteins of intact cells, 2D gel electrophoresis, autoradiography) in response to thyrotropin, phorbol esters and epidermal growth factors are determined.

Results

The model developed for dog thyroid cells has been applied to human cells. 26 primary cultures have been carried out. When seeded in a 1% serum-supplemented medium, thyroid follicles released by collagenase/dispase digestion developed as a cell monolayer that responded to TSH by rounding up and by cytoplasmic retraction. When seeded in serum-free medium, the cells remained associated in dense aggregates surrounded by few slowly spreading cells. In the latter conditions, the cells responded to TSH and other stimulators of cAMP production, such as cholera toxin and forskolin, by displaying very high iodide-trapping levels. Exposure to serum irreversibly abolished this differentiated function. TSH stimulated the proliferation (as shown by DNA content per culture dish) of 1% serum cultured cells (doubling times were reduced from 106 to 76h) and increased by 100% the ^{3}H thymidine labeling indices. In serum-free cultured cells (dense aggregates or cell monolayers after initial seeding with serum) control levels of DNA synthesis were lower, and up to 8-fold stimulation of DNA synthesis occurred in response to 100 mU/ml TSH (stimulation was consistently detected with 20 mU/L), based on measurements of ^{3}H thymidine incorporation into acid-precipitable material and counts of labeled nuclei on autoradiographs (up to 40% labeled nuclei within 24h). The mitogenic effect of TSH required a high insulin concentration (8.3×10^{-7} mol/L) or a low insulin like growth factor I

concentration. The mitogenic effects of TSH were mimicked in part by cholera toxin, forskolin, and dibutyryl cAMP. Epidermal growth factor and phorbol myristate esters also stimulated thyroid cell proliferation and DNA synthesis, but they potently inhibited TSH-stimulated iodide transport. The patterns of protein synthesis induced by TSH and cyclic AMP on the one hand and epidermal growth factor and phorbol esters on the other hand are different. Thyroperoxidase is induced by the first and repressed by the second type of pathway.

Discussion

We conclude that TSH, acting at least in part through cAMP, is a potent growth factor for human thyroid cells and thus provide an experimental basis in vitro for the well established in vivo goitrogenic action of TSH. As in dog thyroid TSH, through cyclic AMP, stimulates both proliferation and differentiation expression, while epidermal growth factor and phorbol esters activate proliferation but repress differentiation expression.
This work represents the first development and characterization of a system of differentiated human thyroid cells in culture. Such a system offers the first experimental model for the study of in vitro transformation of human thyroid cells. On the other hand the number of possible divisions should be increased to about 10 for allowing the system to be used for radiobiological work.

5. DEFINITION OF CELL KINETICS OF THE HUMAN THYROID

Methods

In vivo and in vitro labelling of dog thyroid with ^{3}H methylthymidine and bromodeoxyuridine respectively. In vitro labeling of slices of human thyroids with ^{3}H thymidine for 1 hour. Counting of labeled nuclei by autoradiography and histochemical staining of bromodeoxyuridine. Measurement of labeling index.

Results

Cell population kinetics were studied by bromodeoxyuridine histochemical and ^{3}H thymidine radioautographic labelling in dog thyroids. In vivo labelling with BrdU and in vitro labeling of in vitro incubated slices with ^{3}H thymidine gave similar results (4.10^{-4} to 7.10^{-4}). This validates the use of in vitro labelling of slices for the study of cell kinetics in the thyroid. Thyroid of nine adults, one adolescent girl have been studied by ^{3}H methylthymidine labeling in vitro.

In vitro labelling of human thyroid slices demonstrated a labelling index of 14.8×10^{-5} for follicular cells, of 27×10^{-5} for stromal cells; assuming an S phase of 8 hours, this corresponds to a turnover time of the order of 10 years for the follicular cells and 4 years for the stromal cells. These results show for the first time that human thyroid cells divide about five times during adulthood and therefore that the steady state level of thyroid cell mass results from a balance between cell division and cell loss. A higher turnover time was found as expected in the thyroid of an adolescent (one half year).
5 adenomas have been studied. The labelling index is always higher than in normal tissue (59/83500). Calculation of the doubling time of adenomatous tissue (0.7 year) suggests that several years are necessary to produce a 10^{9}cells (1 g) nodule.

Discussion

The results show unambiguously that adult follicular cells divide albeit very slowly (5 to 6 times during adult life). The rate of renewal is much faster (turnover time = about 6 months) in adolescents. These data explain the long delay between irradiation and both thyroid insufficiency and cancer in man. They constitute the quantitative basis for the evaluation of hypotheses on the pathogeny of thyroid disorders in irradiated patients.

6. HUMAN THYROID TUMORS IN VIVO : DEFINITION OF CELL REGULATION ALTERATIONS; SEARCH FOR ONCOGENES AND GENE REARRANGEMENTS

A) Oncogenes in human thyroid tumors

Methods

- Transfection of NIH $3T_3$ cells by cloned DNA from EJ cells (human bladder carcinoma) and DNA from human thyroid tumors;
- DNA was purified from 16 samples from 13 patients with thyroid tumors. At least 100 ug DNA of each tumor was tested (from Dr E. Fragu, Institut de Recherches sur le Cancer, Villejuif);
- Detection of a human Alu sequences in transformation foci.

Results

The methodology for isolation of transformed foci by cloned DNA from EJ cells has been set up in the laboratory. Several DNA probes have been tried for the demonstration by Southern blotting of human Alu sequences in such foci. Only "blue 8" probe was sufficiently discriminating. 250 foci were isolated among which 126 were tested for the presence of Alu sequences. Only foci obtained with DNA from a lymph node invaded by a follicular cancer metastasis were positive. This has been confirmed by a second transfection with the same DNA. The transforming potency of this DNA is low (0.009 foci/mg DNA) vs. the potency of EJ DNA (0.10 foci/ng DNA). Three successful separate transfection with the cancer DNA but with a very low potency (0.009 foci/mg DNA) vs EJ.DNA (0.10 foci/mg DNA). Contrary to EJ, no success in getting second transfection with DNA extracted from foci (3 trials) was obtained.

Discussion

The methodology of transfection has been set up satisfactorily. The detection of oncogenicity in only one DNA of an advanced metastatic lesion compares with results on other cancer types. It is however disappointing with regard to the efficiency of the assay for oncogene detection. Although the present methodology has been sufficient to demonstrate oncogenicity in DNA, its power does not allow the characterization of the positive DNA.
Our failure is now explained by results by the group of Williams (Cardiff). For this group, passage in vivo in nude mice, a technology not available in our group is necessary for oncogene amplification and isolation. In this study, mutations in cRas protooncogene have been found in most malignant human thyroid tumors.

B) Search for DNA rearrangement around the thyroglobulin gene in thyroid cancer

Methods

Southern blotting of DNA from human thyroid cancers obtained from E.Fragu (Villejuif) exploring Tg gene and regions 10 Kb upstream and downstream from the gene.

Results

In 10 samples, no rearrangement has been found.

Discussion

cMyc and cFos protooncogenes exist on the same arm of chromosome 8 as thyroglobulin gene. The promoter of thyroglobulin is very strong in thyroid cells. By analogy with results on lymphomas, it can therefore be hypothesized that rearrangements leading to the control of cMyc, cFos or other protooncogenes by thyroglobulin promoter can be a cause of thyroid cancer.
The results do not substantiate the hypothesis that thyroid cancer might result from the translocation of a protooncogene under the control of Tg promoter.

C) Definition of cell regulation alterations in autonomous nodules : biochemical mechanisms of autonomy

Methods

a) Measurement of cyclic AMP, and cyclic AMP response to TSH and other hormones; of iodide transport (T/M) and iodide binding to proteins in slices of autonomous nodules and the quiescent normal counterpart tissue. ^{32}P phosphate labelling of slices from human autonomous nodules and contralateral quiescent tissue from surgical specimens. 2D electrophoresis of proteins and autoradiography.

b) Study of the PI response : incubation of slices of normal and adenomatous tissue with ^{3}H inositol for 4 hours to label phosphatidylinositols. After washing, the slices are treated with thyrotropin then extracted with HClO4. Inositol phosphates derivatives are separated on Dowex X50 columns and by HPLC.

Results

The in vitro characteristics of iodide and cyclic AMP metabolism have been compared in tissues from autonomously functioning thyroid nodules and their quiescent counterpart to test the hypothesis that autonomy may result from constitutive activation of the tissue's thyrotropin (TSH), cyclic AMP, protein phosphorylation regulatory axis. As in vivo, nodular tissue took up more iodide. This effect was entirely due to increased transport capacity, the affinity of iodide transport and the fractional binding of iodide to protein remaining unchanged. However, at high concentrations total iodide binding to protein was of the same order in quiescent and nodular tissue. In both tissues, this metabolic step was enhanced by phorbol esters and ionophore A23187. As evaluated by autoradiography of 2D gel protein electrophoregrams, no marked differences in the patterns of protein synthesis or phosphorylation between quiescent and nodular tissue was observed. Basal cyclic AMP levels were similar in

quiescent and nodular tissue. The cyclic AMP response to TSH was lower in nodular tissue, with no change in sensitivity or kinetics; both tissues responded to forskolin. No systematic suppression of iodide inhibition or abnormal response to other hormones or neurotransmitters was found. Three proteins (24K-1, 24-2, 26K) were phosphorylated only in the presence of TSH or forskolin, in both quiescent and nodular tissue. One protein substrate (20K) was phosphorylated in the presence of TSH in the quiescent but not in the nodular tissue. In conclusion : 1) slices from autonomous thyroid nodules reproduce the in vivo characteristics of the lesion and are therefore a suitable in vitro experimental model for biochemical studies ; 2) taken together with data from transplantation experiments, the reproduction in vitro of its in vivo characteristics suggest an inherent defect in the nodule; 3) the homogeneity of biochemical findings within each nodule is compatible with the clonality of the lesion; 4) the autonomous nodule is a minimal deviation tumor; 5) the characteristics of the TSH, cyclic AMP,protein phosphorylation cascade are qualitatively normal. Autonomy does not result from constitutive activation of this sytem; 6) A 20K protein, not phosphorylated in response to TSH in the nodule, could represent an absent negative controlling element.

Phosphatidylinositol response of thyroid adenomas : slices of 6 autonomous adenomas have been compared to slices of their normal quiescent counterpart. In each case thyrotropin has stimulated inositol phosphate generation and cyclic AMP in the normal cells. This demonstrates a dual regulation by TSH in human tissue. In adenomatous slices, TSH normally activates adenylate cyclase but not phospholipase C. Thyroid stimulating immunoglobulins enhance cyclic AMP accumulation but not inositol phosphate generation in slices from 5 normal thyroids.

Discussion

1) Human thyroid tissue is regulated by TSH by the cyclic AMP system and the phosphatidylinositol calcium diacylglycerol cascade (dual control). Thyroid stimulating immunoglobulins which are responsible for hyperthyroidism and Graves disease only stimulate the former system. Autonomy does not result from a higher sensitivity of the abnormal tissue to TSH. On the other hand, two fundamental anomalies have been found; 1) the absence of phosphorylation of one protein in response to cyclic AMP. This suggests that autonomy may result from inactivation (by mutation ?) of a phosphorylated feedback protein inhibitor of the cyclic AMP system.

2) A deficient PI response to TSH in autonomous nodules. The results suggest that thyroid hyperplasia coupled to hyperfunctioning, such as occurs in thyroid adenoma and in Graves disease, is caused by an imbalance between the cyclic AMP and the phosphatidylinositol Ca^{++} regulatory pathways. This hypothesis could apply to hyperfunctioning adenomas of other tissues.

7. DEFINITION OF THE EPIDEMIOLOGY OF X RAY INDUCED HUMAN THYROID TUMORS

COOPERATIVE EPIDEMIOLOGICAL STUDY OF IRRADIATED PATIENTS

Methods

A) X Ray

A common protocol for the retrospective study of patients irradiated by X rays for therapeutic purposes has been initiated in Brussels, Pisa,

Villejuif and Leyde. Both a retrospective study of already investigated patients and a recall study have been initiated. Data are collected and centralized in Brussels.

B) Radioiodine

Register of all patients having received high diagnostic doses of ^{131}I (50 to 200 uCi) at Bordet Institute between 1955 and 1970. Patients having no apparent thyroid disease are recalled and examined

Results

a) X ray irradiated patients : the data corresponding to 252 patients and 127 controls from Pisa and 242 patients from Villejuif have been introduced. Additional controls from Pisa, and controls from Villejuif have been requested. No data have been obtained from Leyde.

b) Patients irradiated by ^{131}I for diagnostic purposes. 9437 records have been obtained. 685 patients satisfied the criteria (of dose and absence of thyroid disease). 338 have been recalled by letter. Only 19 patients came to the clinics; 2 had nodules.

Discussion

a) Results of the retrospective study on the consequences of X ray irradiation are difficult to get. This program which depends on data gathering in other centers is slower than foreseen.

b) Results of a pilot retrospective study on normal patients having received I^{131} for diagnostic purposes are disappointing. The recall rate of these patients is much too low to allow the collection of significant data.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

M. PAVLOVIC, INSERM, Hôpital Bicêtre, Paris, France
S. BEEBE , Dept Physiology, Vanderbilt University, Nashville, USA
P. DOR , Chirurgie, Institut Bordet, Bruxelles, Belgique
P. FRAGU , Institut du Cancer, Villejuif, France
A. PINCHERA, Centro Endocrinologia, Pisa , Italia
F. MALONE , Institute of Technology, Dublin, Irlande

V. Publications:

- DUMONT, J.E., ROGER, P., SERVAIS, P., GERARD, C., LAMY, F., LECOCQ, R., VAN HEUVERSWYN, B., VAN SANDE, J., VASSART, G., MOCKEL, J. Regulatory networks involved in the control of thyroid follicular cell function, proliferation and differentiation : the model of the dog thyroid. In "Thyroglobulin - The Prothyroid Hormone" (Progress in Endocrine Research and Therapy, vol 2) M.C. Eggo and G.N. Burrow, eds, Raven Press, New York, pp 283-296, 1985.
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- REUSE, S., ROGER, P.P., VASSART, G., DUMONT, J.E. Enhancement of cMyc concentration in dog thyrocytes initiating DNA synthesis in response to thyrotropin, forskolin, epidermal growth factor and phorbol myristate ester. Biochem. Biophys. Res. Commun. 141 n° 3, 1066-1076, 1986.

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- DUMONT, J.E., ROGER, P., LUDGATE, M., Autoimmunity and thyroid growth : methods, concepts and misconceptions. Acta Endocrinol. (Copenh.), suppl. 281, 299-301, 1987.
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- ROGER, P.P., RICKAERT, F., HUEZ, G., AUTHELET, M., HOFMANN, F, DUMONT J.E., Microinjection of catalytic subunit of cyclic AMP-dependent protein kinases triggers acute morphological changes in thyroid epithelial cells. FEBS Lett., 232(2), 409-413, 1988.
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RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-080-D

Gesellschaft für Strahlen-
und Umweltforschung mbH
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. W. Gössner
Institut für Pathologie
GSF
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Telephone number: 089-3187 2312

Title of the research contract:

Pathogenesis of late somatic effects of radiation.

List of projects:

1. Radiation-induced oncogenesis under different exposure conditions.
2. Pathogenesis of radiation-induced cancer.

Title of the project no.: 1

Radiation-induced oncogenesis under different exposure conditions

Head(s) of project:

W. Gössner

Scientific staff:

W. A. Müller, U. Linzner, A. Luz, A.B. Murray, E. Schäffer

I. Objectives of the project:

To study the modification of dose dependence in radiation-induced oncogenesis resulting from different radionuclides, paying particular attention to the effects of different qualities of radiation, radiation dose rates and time patterns. The effects at low irradiation doses are of special interest.

II. Objectives for the reporting period:

a) Evaluation of malignant lymphoma/leukaemia in experiments with short-lived bone-seeking radionuclides applied at different age.
b) Study of the influence of age at incorporation for bone tumour induction by the short-lived ^{227}Th in CBA mice, which are less sensitive for bone tumour induction than NMRI mice.
c) Investigation of the effect of local (parosteal) injections of colloidal ^{227}Th and ^{228}Th solutions. Distribution and dosimetry estimations, as well as long-time pilot studies, with small groups of animals.

III. Progress achieved:

1. METHODOLOGY

a) The frequency of malignant lymphoma/leukaemia (ML) in previous experiments with incorporation of ^{227}Th in female NMRI mice of different age was evaluated (37 kBq/kg corresponds to 200 cGy mean skeletal dose).

b) Female CBA mice received i.p. 37 or 185 kBq/kg ^{227}Th at age of 3.5 or 18 months. There were 43/50 animals in the younger age group and 98/97 animals in the older age group. In addition 50 untreated animals of corresponding age were observed. Tumour incidence was corrected for competing risk and evaluated up to ten surviving animals. Differences were tested with a log rank test.

c) Colloidal solutions of ^{227}Th and ^{228}Th were injected locally at the tibia of 12-week-old female NMRI mice. Three dose groups were formed from each thorium isotope: (^{227}Th: 3/9/ 25 kBq/mouse; ^{228}Th: 1/3/ 9 kBq/mouse). Each group consisted of 20-25 animals.

2. RESULTS

a) Malignant lymphoma/leukaemia

Incorporation at 1 month of age

The following significant effects were found for the rate of ML at 601-800 days of age.

control 8 % (3/38), 74 kBq/kg 38 % (9/24) p = 0.0058
148 kBq/kg 37 % (10/27) p = 0.0049

No significant induction of ML was found after incorporation of 18.5, 37 or 185 kBq/kg ^{227}Th.

Incorporation at 3 months of age

The following significant effect was found for the rate of ML at 201-400 days of age.

control 3 % (3/99), 185 kBq/kg 17 % (16/94) p = 0.0009

No significant induction of ML was found after incorporation of 18.5 or 74 kBq/kg ^{227}Th respectively.

Incorporation at 18 months of age

No significant induction of ML was found after incorporation of 37 or 185 kBq/kg.

Incorporation of 2 fractions of 37 kBq/kg

Incorporation at 3 and 5 months or 3 and 12 months of age did not induce ML. After incorporation at 12 and 14 months a significant

(p = 0.027) increase of ML rate was observed at 601-700 days of age: 15 % (10/65) versus 2 % (1/42) in controls.

b) Influence of age on tumour induction

Bone sarcoma

A significant difference (p = 0.005) between the two age groups was only observed after incorporation of 37 kBq/kg ^{227}Th (550 days after incorporation):

3.5 month group: 3 % (controls 0 %)
18 month group: 19 % (controls 2 %)

Malignant lymphoma/leukaemia

Induction was only significant in the older age group.

18 month group, 350 days after incorporation:

37 kBq/kg 12 % (controls 2 %) p = 0.023
185 kBq/kg 20 % p = 0.025

c) Distribution studies showed that 95 % of the thorium isotopes stayed at the injection site, whereas the radium daughters were distributed throughout the skeleton in a similar way as after protracted i.p. injected radium.

The first results of the pilot studies showed high systemic osteosarcoma incidence (ca. 50 %) for the middle doses (9 kBq/mouse for ^{227}Th and 3 kBq/mouse for ^{228}Th) and a few local osteosarcomas in the low dose groups (3 kBq/mouse for ^{227}Th and 1 kBq/mouse for ^{228}Th).

3. DISCUSSION

a) The results show that there is no simple law for dose dependence of induction of ML. In addition dose-relationships may be different for growing and adult animals.

b) Female CBA mice, which are significantly less sensitive for bone sarcoma induction than NMRI mice, show the same higher sensitivity for bone sarcoma induction at older age as NMRI mice, but also only in the lower dose range (200 cGy). The induction of malignant lymphoma in animals 18 months of age is surprising, since it was not observed in NMRI mice (which have a higher burden of spontaneous malignant lymphoma/leukaemia).

c) The injection method applied seems to be suited for radium protraction experiments in place of repeated injection with short-lived ^{224}Ra in long-term experiments with very low doses.

IV. Objectives for the next reporting period:

a) Start of experiments with very low ^{224}Ra-producing ^{228}Th doses (ca. 40 Bq per animal) with different mice strains for lymphoma induction.
b) Tumour induction after incorporation of 1.85 kBq/kg ^{227}Ac in 3 month old mice and ^{227}Th in 4 month or 12 month old mice.
c) Distribution and dosimetry studies after parosteal injection of further alpha- and beta-emitting radiocolloids (^{210}Po, ^{95}Zr and ^{106}Ru) for low dose protraction experiments.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

LUZ, A., MÜLLER, W. A., LINZNER, U., MURRAY, A. B., WICK, R. R., GÖSSNER, W.:
Die Zeit als Kofaktor des Strahleninduzierten Knochentumors.
In: Fortschritte der Osteologie in Diagnostik u. Therapie (Hrsg.: F.H.W. Heuck, E. Keck). Berlin-Heidelberg: Springer Verlag, 119-125 (1988)

MÜLLER, W. A., LINZNER, U., LUZ, A.:
Early induction of leukemia (malignant lymphoma) in mice by protracted low alpha doses.
Health Physics 54, 461-463 (1988)

Title of the project no.: 2

Pathogenesis of radiation-induced cancer

Head(s) of project:

W. Gössner

Scientific staff:

V. Erfle, J. Schmidt, P. G. Strauß, A. Luz, A. B. Murray, A. Schön

I. Objectives of the project:

The evaluation of the molecular mechanism responsible for radiation carcinogenesis.
Investigation of exogenous agents which might modify the development and progression of radiation-induced tumours.

II. Objectives for the reporting period:

a) Further studies on the expression of c-fos, c-myc and v-fos oncogenes in "in vitro" mouse mandibular condyles after transformation and induction of an "in vitro" osteosarcoma-like osseous lesion by FBR osteosarcoma virus.
b) Further studies on the transcriptional activity of retroviral LTRs in osteogenic cells.
c) Final evaluation of an experiment studying the effect of ciclosporine on ^{227}Th-induced oncogenesis.

III. Progress achieved:

1. Methodology

a) Northern blot analysis was carried out to discriminate between endogenous c-fos mRNA and exogenous v-fos transcripts in FBR osteosarcoma virus-infected mandibular condyles and in the transplant tumours. In situ hybridizations with fos and myc probes were performed to determine which cells of the tissues were expressing these oncogenes.
b) The transcriptional activity of retroviral LTRs was measured by CAT-assays, using CAT-vectors containing various retroviral LTRs.
c) Female NMRI mice, 85 days old, received i.p. 37 kBq/kg ^{227}Th (mean skeletal dose 2 Gy). Two groups received weekly injections of ciclosporine at age 197-386 days with a dosage of 10 or 50 mg/kg. There were about 50 mice in each group.

2. Results

a) Virus-derived v-fos transcripts were occasionally detected in FBR osteosarcoma virus-infected mandibular condyles up to 2 weeks in culture. Low expression of v-fos mRNA was detected after transplantation of the transformed condyle in the transplant tumour tissue. The oncogene was expressed at a high frequency, however only in a few distinct areas of the tumour. In situ hybridization carried out on cryo-sections of virus infected and of non-infected control condyles showed high c-fos expression in a few cells in the basal area of the tissue. The expression of v-fos was not followed by c-myc RNA synthesis.
b) The transcriptional activity of the LTRs of RFB osteoma virus and of the osteoma-derived OA murine leukaemia virus (OA MuLV) did not change during osteogenic differentiation of ROS 17/2.8 cells in vitro. LTR activity was stimulated 2-fold in undifferentiated ROS 17/2.8 cells by dexamethasone and by retinoic acid.
c) Immunosuppression by treatment with ciclosporine (C) did not influence the frequency of osteosarcoma after incorporation of 37 kBq/kg ^{227}Th. There was also no change in either the latency,

the multiplicity, or the metastasis of radiation-induced osteosarcoma. The only effect of combined treatment was an additional induction of lung tumours - corrected incidence after 750 days:
Controls 13 %,
37 kBq/kg ^{227}Th 16 %, 50 mg/kg group C 15 %
^{227}Th plus C 29 % ($p < 0.05$, log rank test).

3. Discussion

a) The FBR osteosarcoma virus induced tumours were heterogenous with respect to fos oncogene-expression. The fos transcripts were only found in a few cells of distinct areas. These results indicate that v-fos-expressing cells may secrete factors which induce proliferation of surrounding cells. The high expression of c-fos seen in some cells of uninfected condyles in culture suggests a role of c-fos in the initial steps of osteogenic differentiation.

b) The LTRs of bone tumour-derived retroviruses show transcriptional activity upon transfection into osteogenic cells. The LTR activities can be modulated by various factors, such as dexamethasone or retinoic acid. However, the state of osteogenic differentiation of the host cells does not appear to have an effect on the LTR activity. Hence, structural differences of the LTRs of different bone tumour-derived retroviruses do not account for their different pathogenicity. Other sequences of the retroviral genome should be considered as determinants of the biological effects of these viruses.

c) The lack of influence of immunosuppression on the development of radiation-induced osteosarcoma corresponds with the low antigenicity of osteosarcomas. The induction of lung tumours by the combined treatment with ^{227}Th and ciclosporine is difficult to understand.

IV. Objectives for the next reporting period:

1) Further studies on the expression of structural and bone cell-specific genes and cellular oncogenes in "in vitro" differentiating osseous tissue and after neoplastic transformation by radiation-activated retroviruses.
2) Studies on the effect of RFB osteoma virus on the differentiation and transformation of primary and permanent osteogenic cells in vitro.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Department of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Aarhus, Denmark (Dr. F. S. Pedersen)

Studiecentrum voor Kernenergie, SCK/CEN, Boeretang 200, B-2400 Mol, Belgium (Dr. M. Janowski)

The Rappaport Family Institute for Research in the Medical Sciences, Technion-Israel Institute of Technology, Haifa, Israel (Prof. Dr. M. Silbermann)

VI. Publications:

Schmidt, J., Luz, A., Erfle, V.:
Endogenous murine leukemia viruses: frequency of radiation-activation and novel pathogenic effects of viral isolates.
Leukemia Res. 12, 393-403 (1988)

Strauß, P. G., Schmidt, J., Pedersen, L., Erfle V.:
Amplification of endogenous proviral MuLV sequences in radiation-induced osteosarcomas.
Int. J. Cancer 41, 616-621 (1988)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-083-D

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Prof. Dr. A.M. Kellerer*
Prof. Dr. H. Spiess*

Telephone number: 089-3187 -2312

Title of the research contract:

Epidemiological studies of radiation carcinogenesis and its biophysical basis.

List of projects:

1. Late effects in Ra-224 treated ankylosing spondylitis patients.
2. Late effects in Ra-224 treated juvenile and adult patients.
3. Epidemiology of radiation carcinogenesis.

*This research programme is carried out in coordination with the "Institut für Medizinische Strahlenkunde der Universität Würzburg", Prof. Dr. A.M. Kellerer and the "Kinderpoliklinik der Universität München", Prof. Dr. H. Spiess.

Title of the project no.: 1

Late effects in Radium-224 treated ankylosing spondylitis patients

Head(s) of project:

Prof. Dr. W. Gössner

Scientific staff:

Dr. R. R. Wick, Dr. W. A. Müller

I. Objectives of the project:

All three projects in this research programme are aimed at epidemiological studies of radiation effects in patients injected with Ra-224.

Project 1 is concerned with more than 1500 ankylosing spondylitis patients treated between 1948 and 1975 with repeated intraveneous injections of Ra-224. The alpha-doses to the skeleton, on average 0.67 Gy, are considerably lower than the doses in the earlier patients studied in Project 2. The causes of death, and occurrence of other lesions possibly related to the Ra-224 treatment, are analysed and compared with results in a control group of ankylosing spondylitis patients not treated with radioactive drugs.

II. Objectives for the reporting period:

Contact and follow-up of patients of the exposure group and the control group. Registration of causes of death. Comparison of results in the exposure and control groups and evaluation with respect to the risk of bone tumours, leukaemias, kidney and liver diseases, and other diseases known, or supposed from Project 2, to be related to the Ra-224 treatment.

III. Progress achieved:

This study includes most patients treated for ankylosing spondylitis since about 1948 in the Federal Republic of Germany with minor amounts of Ra-224 and not yet followed in Project 2. By December 1988 our study consists of 1473 ankylosing spondylitis patients from 9 hospitals. These patients have been collected from all orthopaedic hospitals in the Federal Republic of Germany and West Berlin known to have treated notable numbers of patients with Ra-224.

In addition there exists a control group of 1336 ankylosing spondylitis patients not treated with radioactive drugs in order to provide comparative information on causes of death and lesions possibly related to the basic disease itself or to chemotherapy. Current follow-up has shown that a substantial part of the patients of the original exposure and control groups have been treated with X-rays previously. These patients have been deleted from the original groups by end of this year.

Until end of 1988, 499 patients in the exposure group and 616 patients in the control group have died (Table 1). Causes of death have been ascertained in 486 patients in the exposure group and in 546 patients in the control group. Table 2 shows the skeletal and soft tissue diseases observed so far. In this table we restricted to those diseases which are known or supposed from Project 2, the higher dose study, to be associated with a former administration of Ra-224.

Until now, three cases of tumours in the skeleton have been recorded. Two of the three cases were tumours of the bone marrow, while in Project 2 mostly osteosarcomas were observed. According to the age dependent spontaneous rates for bone tumours in the Federal Republic of Germany from data in the calendar years 1950 to 1978 the expected number of spontaneous bone tumours for the present follow-up time is 0.4 - 0.7 cases for this study group. Assuming a Poisson distribution, the probability is 0.034 for an occurrence of 3 or more cases when the expectation is 0.7 cases. In the control group only one case of skeletal tumour has been observed until now. This is within the limitations of expected cases for that group.

Diseases of haematopoietic tissue among living and dead patients included: bone marrow failure (10 in the exposure group vs. 7 in the control group) and leukaemias (7 cases vs. 4). In the exposure group three of the leukaemias were of the chronic myeloid type and there was only one of the acute lymphoblastic type, whereas no case of chronic myeloid, but three cases of lymphoblastic leukaemias were observed in the control group. This apparent difference is somewhat parallel to the observation that two of the three skeletal tumours are bone marrow tumours, whereas much less had been related to the bone marrow in the higher dose study of Project 2. Certain disorders of the haematopoietic system following treatment with Ra-224 were observed earlier by other authors even at the same low dose level.

Table 1: Follow-up status of ankylosing spondylitis patients in the exposure and control groups (Dec. 1988)

	Exposure group	Control group
Total number of patients	1579	1461
Treated with X-rays additionally	106	125
Remaining patients	1473	1336
Deceased patients	499	616
Death cause certified	486	546
Cause of death not yet known, still in work	13	70

Table 2: Skeletal and soft tissue diseases (Dec. 1988)

	Exposure group	Control group
Malignant skeletal tumours	3	1
Exostosis	0 (+ 1L*)	1
Bone marrow failure	4 (+ 6L)	5 (+ 2L)
Leukaemias	6 (+ 1L)	4
Acute leukaemias	1	4
Acute lymphoblastic leukaemias	1	3
Chronic leukaemias	5	0
Chronic myeloid leukaemias	3	0
Type unspecified	0 (+ 1L)	0
Total cancers	79 (+14L)	114 (+12L)
Kidney diseases	55 (+68L)	64 (+41L)
Liver diseases	21 (+47L)	31 (+14L)
Cataracts	3 (+16L)	1 (+12L)

*L = living

IV. Objectives for the next reporting period:

The follow-up of patients in the exposure and control groups will be continued and the results evaluated with special regard to the late effects in bone, haematopoietic tissue, kidney, liver, and other organs known or supposed from Project 2 to be affected by injected Ra-224. The patients of the exposure group will be addressed with a new questionnaire compiled together in close cooperation with Project 2.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

10 Orthopaedic and Rheumatic hospitals from the F. R. Germany and West Berlin.
Close cooperation with the working groups in Projects 2 and 3 of this contract.

VI. Publications:

WICK, R. R., GÖSSNER, W.:
Recent results of the follow-up of Radium-224 treated ankylosing spondylitis patients.
Brit. J. Radiol. (in press)

Title of the project no.: 2

Late effects in Radium-224 treated juvenile and adult patients

Head(s) of project:

Prof. Dr. H. Spiess

Scientific staff:

Prof. Dr. M. Jensen, K. Kogler

I. Objectives of the project:

Follow-up initiated in 1948 of patients treated with Ra-224 as juveniles or adults.
Determination of stochastic and non-stochastic radiation effects and their dose, time, and age dependence.

II. Objectives for the reporting period:

Cooperation with Prof. C. W. Mays (National Cancer Institute/NIH, Radiation Epidemiology Branch, Bethesda/MD, USA) in the cataract and kidney study.

Review of existing data.

Cooperation with Prof. F. Stefani (Eye Hospital of the University of Munich).

III. Progress achieved:

At 3 year intervals we are following the health of 900 patients (509 men, 173 women, 111 boys, and 107 girls) who received repeated injections of Ra-224 after World War II, mostly for treatment of ankylosing spondylitis or bone tuberculosis, but also for other non-cancerous diseases.
The calculated alpha-doses to the marrow-free skeleton ranged from 0.06 - 58 Gy (average: 4 Gy). At the time of last follow-up, 517 patients had died. 56 bone cancers were reported, 94 soft-tissue malignancies were observed.

The frequency of the following three cancer types was considered to be elevated:
breast: 15 cases observed vs. 3.8 - 5.6 cases expected;
liver: 5 cases observed vs. 1.0 case expected;
kidney: 5 cases observed vs. 2.2 - 2.4 cases expected.

Breast tissue is known to be sensitive to radiation-induced cancer, especially when the radiation is received at young age. In the patients injected as girls at an age of 2-20 years, 8 breast cancers have appeared vs. 0.4 - 0.6 cases expected.
No significant excess of other cancers has been observed among the Ra-224 patients, including stomach (11 cases observed vs. 7-9 cases expected), intestine and rectum (7 obs. vs. 8-9 exp.), lung (15 obs. vs. 16-17 exp.), skin (3 obs. vs. 4-6 exp.), bladder (4 obs. vs. 2-3 exp.), uterus (4 obs. vs. 3-5 exp.), ovary (2 obs. vs. 1 exp.), prostate (6 obs. vs. 4-6 exp.), and brain (2 obs. vs. 1-2 exp.).

One of each of the following cancer types occurred: parotid gland, oesophagus, follopian tube, thyroid, Hodgkin's lymphoma, multiple myeloma, medullary carcinoma, and peritoneal mesothelioma.
Leukaemia was observed in 6 patients compared to 2 cases expected (based on German National Statistics). However, some of the spondylitis patients took leukaemogenic drugs.

TABLE 1: Summary of the Radium-224 patients in Project 2 (Dec. 1988)

	Age at first injection 1-20 yr	Adult	Total
Traced patients	218	682	900
Patients with ankylosing spondylitis	0	392	392
Patients with TB	218	240	458
Patients with other diseases	0	50	50
Deaths	91	426	517
New deaths since 1987	1	7	8
Unknown cases of deaths	4	22	26
Skeletal diseases:			
Bone sarcoma	37	18	55
Exostosis	29	0	29
Growth retardation	28	0	28
Tooth breakage	40	20	60
Soft tissue diseases:			
Cataract	18	47	65
Leukaemia	1	5	6
Liver (non-cancer)	4	33	37
Kidney (non-cancer)	11	69	80
Diabetes	3	28	31
Cancers of soft tissue:			
Lung	1	13	14
Mamma	8	7	15
Uro-genital region	1	17	18
Stomach	2	17	19
Skin	1	3	4
Liver	1	5	6
Kidney	1	5	6
Others	2	8	10

IV. Objectives for the next reporting period:

Getting in contact with non-responding patients.
Screening of cataract patients at the Eye Hospital by Prof. Stefani.
Looking for possible diseases in the second generation.
Inviting patients for examination.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Prof. Dr. C. W. Mays, National Cancer Institute/NIH, Radiation Epidemiology Branch, Bethesda/MD, USA
- Prof. Dr. F. Stefani, Eye Hospital, University of Munich
- Prof. Dr. Gurland, University of Munich (Großhadern)
- Prof. Dr. E. Sonnabend, Dental Clinique, University of Munich
- Dr. D. Chmelevsky, GSF, Institut für Strahlenschutz, Neuherberg

VI. Publications:

SPIESS, H., MAYS, C. W., CHMELEVSKY, D.:
Malignancies in patients injected with ^{224}Ra.
Brit. J. Radiol. (in press)

Title of the project no.: 3

Epidemiology of radiation carcinogenesis

Head(s) of project:

Prof. Dr. A. M. Kellerer

Scientific staff:

Dr. J. Breckow, M. Lassmann, H. Friede

I. Objectives of the project:

The project aims at the further development of mathematical methods for the analysis of the dose, age, and time dependence of radiation--induced neoplasms. It is equally concerned with the application of these methods to animal studies and to epidemiological investigations. Risk estimates for low doses of ionizing radiations need to be based on a synopsis of essential results obtained from animal studies and from the major human epidemiological investigations. Such a synopsis requires the utilization of comparable mathematical methods and models; the efforts in this project are focussed on this need. The joint analysis of dose, age, and time dependences is particularly important in view of the new risk estimates being obtained after the revision of the Japanese dosimetry on the basis of the extended data. The great public concern about the risks even of small doses have given added urgency to this work.

II. Objectives for the reporting period:

Among a variety of issues there were four topics of special importance in the past reporting period. Two of these issues concerned the data from the follow-up of the Ra-224 patients by Spiess and Mays which is the objective of Project 2. The first problem is the still partly unresolved question of a dose-rate effect in the induction of osteosarcomas in these patients. The second main point of current interest is the emergence of increased incidences of malignancies other than osteosarcomas. Two other major items concern the reestablishment of risk estimates at low doses on the basis of the revised Japanese dosimetry, and the attempt to extend the comparative analysis in BEIR IV to cover also the Czechoslovakian data on uranium miners.

III. Progress achieved:

1. The time factor for osteosarcomas in the Radium-224 patients

The analysis of a possible dependence of bone sarcoma rates on the duration of treatment has shown that results obtained earlier by C. W. Mays were essentially correct, although they were based on less complete data and on more approximative mathematical methods.

A double approach was chosen. First, the presence of the reversed dose-rate effect has been shown in terms of a set of rank-order tests that is applied to the data when they are classified in terms of skeletal dose, treatment duration, and time at risk. Secondly, a quantitative fit has been established in terms of maximum likelihood to the following relation for the cumulative probability for osteosarcomas as a function of the mean skeletal dose D, the duration of treatment T, and the time after treatment t:

$$R(t,D,T) = R_0(t)(D+0.23D^2)\exp(-0.52\ D/T)$$

The mean skeletal dose is expressed in Gray and the duration of treatment, T, in month. The base-line function, $R_0(t)$, resembles the log-normal distribution which has been obtained in earlier analyses.

This result proves that the reversed dose-rate effect, i.e. the increased incidence of osteosarcomas with increasing duration of treatment, is not merely an artefact of the non-linearity of the dose-response relation. Instead there is a true increase of the incidence with increasing "dose rate", D/T, at equal doses. According to this result the dose dependence is linear-quadratic when the treatment time is proportional to dose. When the treatment times increase less than proportionally to dose, the dose dependence is closer to linearity.

2. Other neoplastic diseases in the Radium-224 patients

A recent, important result of the continuing follow-up of surviving Ra-224 patients of Project 2 is the apparent excess in the incidences of several neoplastic diseases (see Project 2). The observed incidences were assessed in terms of population statistics data from Germany which were cross-checked with data from the United States (SEER-tables) and were found to be largely consistent in general trends. In terms of this analysis a significant excess of breast cancers was shown and a somewhat less marked excess of liver and kidney cancers. At this stage no dose-

-effect relations can be deduced, and the results of the continuing follow-up will have to be awaited. Available data emphasize the importance of the continued observation and the need to follow the trends which have now become apparent.

3. Risk estimates from the new data in Hiroshima and Nagasaki

Detailed calculations on the basis of the revised dosimetry and the extended observations will have to await the availability of the entire data base. Nevertheless it was important to perform preliminary analyses from results made available in recent RERF reports. This led to the tentative establishment of factors of change in the risk estimates for radiation-induced cancer mortality; these risk estimates were compiled and critically evaluated in a number of reports. The work was specifically required for application in the study of the feasibility of epidemiological research in West European countries after Chernobyl; this is part of the response of the European Commission to the reactor accident.

4. Cooperation with the epidemiological studies on Czechoslovakian uranium miners

A colleague from the Institute of Hygiene and Epidemiology in Prague joined the work on this project during a research visit. This is intended to be the beginning of a cooperation on the development of mathematical methods, to evaluate the data on the Czechoslovakian uranium miners by methods largely equivalent to those utilized in the BEIR IV report which did not treat the Czechoslovakian data.

A two-step procedure is being followed. In a first step, algorithms are developed that are largely identical to those used in BEIR IV. In a second step, a somewhat more general approach will be implemented. In BEIR IV a simplified model is utilized that provides for a decline in time after exposure of the relative risk factors. The model differs from the usual treatment in non containing a factor that stands for the changing sensitivity with age at exposure; instead there is only a dependence on current age. This can confound the dependence on time after exposure, and it is therefore considered necessary to apply an analysis that includes a dependence on age at exposure.

IV. Objectives for the next reporting period:

The current work on items 2 to 4 will be continued. There will be additional work on the comparative analysis on the entire spectrum of tumours induced by gamma-rays and by neutrons in male Sprague-Dawley rats. This is part of the continued cooperation with Dr. Lafuma and colleagues at CENFAR; it will extend previous work that concentrated on the induction of lung cancers in the Sprague-Dawley rats by radon daughters, neutrons, and gamma-rays.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- GSF, Institut für Strahlenschutz, Neuherberg
- Eye Hospital of the University of Munich
- National Cancer Institute, NIH, Radiation Epidemiology Branch, Bethesda/MD, USA
- CEN, Fontenay-aux-Roses
- Institute of Hygiene and Epidemiology, Prague

VI. Publications:

CHMELEVSKY, D., KELLERER, A.M., LAND, C.E., MAYS, C.W., SPIESS, H.:
Time and dose dependency of bone-sarcomas in patients injected with Radium-224.
Radiat. Environ. Biophys. 27, 103-114 (1988)

CHMELEVSKY, D., MAYS, C.W., SPIESS, H., STEFANI, F.H., KELLERER, A.M.:
An epidemiological assessment of lens opacifications with impaired vision in patients injected with Radium-224.
Radiat. Res. 115, 238-257 (1988)

CHMELEVSKY, D., MAYS, C.W., SPIESS, H., STEFANI, F.H., KELLERER, A.M.:
The cataract response in Radium-224 patients.
Brit. J. Radiol. (in press)

KELLERER, A. M.:
Strahlenexposition während der Schwangerschaft.
In: Prophylaxe in der Schwangerschaft, Stillen und Kinderernährung (Ed.: H. Spiess) Marburg: Deutsches Grünes Kreuz, 111-128 (1988)
ISBN 3-88809-130-6

KELLERER, A. M.:
Cancer mortality in Hiroshima and Nagasaki - Recent implications for the risk estimates.
In: Proc. Intern. Colloquium "Epidemiological Investigations on the Health-Effects of Ionizing Radiation", 31-41, Inst.f.Strahlenschutz, Köln (1988)

KELLERER, A. M.:
Krebsmortalität in Hiroshima and Nagasaki - Neue Risikoschätzungen und ihre Bewertung.
In: Aktuelle Fragen zur Bewertung des Strahlenkrebsrisikos. Veröffentlichungen der SSK, Bd. 12. Stuttgart, New York: Gustav Fischer Verlag, 37-64, 1988

KELLERER, A. M.:
Die neue Bewertung der Strahlenrisiken - Folgerungen aus der Revision der Dosimetrie in Hiroshima und Nagasaki.
In: Die Wirkungen kleiner Dosen. Springer-Verlag (in press)

KELLERER, A. M., BRECKOW, J.:
Neue Erkenntnisse zur Dosisrelation nach der Revision der Dosimetrie in Hiroshima und Nagasaki und Auswirkungen auf den Strahlenschutz.
Proceedings Symp. Medical Physics, Tübingen (in press)

LAFUMA, J., CHMELEVSKY, D., CHAMEAUD, J., MORIN, M., MASSE, R., KELLERER, A. M.:
Lung carcinomas in Sprague-Dawley rats after exposure to low doses of radon daughters, fission neutrons, or gamma-rays.
Radiat. Res. (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Contract no.: BI6-D-221-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. W. Gössner
Institut für Pathologie
GSF
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Prof. Dr. A.M. Kellerer*
Prof. Dr. H. Spiess*

Telephone number: 089-3187 2312

Title of the research contract:

Investigation of the cataract incidence in the German Radium-224 patients.

List of projects:

1. Epidemiological investigation on radiation cataract by the ophthalmological examination of patients who had received Ra-224.

*This research programme is carried out in coordination with the "Institut für Medizinische Strahlenkunde der Universität Würzburg", Prof. Dr. A.M. Kellerer and the "Kinderpoliklinik der Universität München", Prof. Dr. H. Spiess.

Title of the project no.: 1

Epidemiological investigation on radiation cataract by the ophthalmological examination of patients who had received Radium-224

Head(s) of project:

Prof. Dr. H. Spiess

Scientific staff:

Prof. Dr. F. Stefani

I. Objectives of the project:

A serial examination provides a possibility to detect cataracts at an early stage where they can be distinguished clinically from spontaneous cataracts or cataracts associated with the original disease that led to the radium treatment. An important feature of this ophthalmologically oriented follow-up will be the repeated examination at regular time intervals of the same patients to assess the evolution of the radiation-induced cataracts. The aim is to gain further insight into the time and dose dependences.

II. Objectives for the reporting period:

Continuing the examinations of patients below 50 years of age.

Sent some patients to Prof. Dr. Hockwin (Eye Hospital Bonn) for screening them with a "Scheinpflugkamera".

Preparing the paper "Subcapsular cataracts in patients injected with a solution of Radium-224, colloidal platinum, and the red dye Eosin ("Peteosthor").

III. Progress achieved:

This is a summary of reports based on 218 patients of the Spiess series treated as juveniles with "Peteosthor" at age of 20 years or younger. 89 of these patients have died, 129 patients are still alive. 34 patients had died from bone sarcoma (30 osteosarcomas, 4 chondrosarcomas), predominantly during their growth period. An address of the ophthalmologists was known of 65 of the 129 living patients.
Nine of this group were examined at the Eye Hospital of the University of Munich. The examinations included visual acuity tests, measurements of the i.-o. pressure, biomicroscopy of the lens, retroillumination of the lens, transillumination of the iris, ophthalmoscopy, photographic documentation of the lenses, and biometry of the lens using Standardized Echography.

IV. Objectives for the next reporting period:

Collection of further diagnosis:
a) from ophthalmologists of the patients
b) additional examinations in the Eye Hospital Munich

Screening of cataract patients at the Eye Hospital of the University Bonn by Prof. Dr. Hockwin.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. Dr. C. W. Mays, National Cancer Institute/NIH, Radiation Epidemiology Branch, Bethesda/MD, USA

Dr. D. Chmelevsky, GSF, Institut für Strahlenschutz, Neuherberg

Prof. Dr. Hockwin, Eye Hospital, University of Bonn

VI. Publications:

CHMELEVSKY, D., MAYS, C.W., SPIESS, H., STEFANI, F.H., KELLERER, A.M.:
Dose dependence for cataracts in Radium-224 patients.
Brit. J. Radiol. (in press)

STEFANI, F.H., SPIESS, H., MAYS, C.W.:
Subcapsular cataracts in patients injected with a solution of Radium-224, colloidal platinum, and the red dye Eosin ("Peteosthor").
Brit. J. Radiol. (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Contract no.: BI6-D-085-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. U. Hagen
Institut für Strahlenbiologie
GSF
Ingolstädter Landstr. 1
D - 8042 Neuherberg

Telephone number: 89-3187 2250

Title of the research contract:

Molecular and cellular mechanisms of neoplastic cell transformation.

List of projects:

1. Molecular and cellular mechanisms of neoplastic cell transformation.

Title of the project no.: BI 6 - 085 - D

Molecular and cellular mechanisms of neoplastic cell transformation

Head(s) of project:

Prof. Dr. Ulrich Hagen
Institut für Strahlenbiologie der GSF
D-8042 Neuherberg

Scientific staff:

Dr. Cornelia Morawetz	(100 %)
Dipl. Biol. Wolfgang Vogel	(100 %)
Prof. Dr. Ulrich Hagen	(25 %)
Prof. Dr. Klaus-Rüdiger Trott	(15 %)

I. Objectives of the project:

Among other mechanisms for radiation induced cell transformation, the transposition (translocation) of oncogenes and the movement of viral enhancer elements have been discussed. As a model for the inducibility of transposition of mobile gene elements the behaviour of Ty-elements in yeast cells will be studied after treatment with mutagenic agents. In addition, cell transformation of mammalian cells (C3H/T1/2) will be tested in respect of the dose effect relationships as modified by fractionation, low dose rate and combined modality with chemicals.

II. Objectives for the reporting period:

a) The strain specificity of induced transposition was tested as well as the mechanisms of Ty insertion into plasmids. Studies on plasmids with Ty elements allow to determine the specific nucleotide sequence around the locus of Ty insertion and possible rearrangements.

b) Repair kinetics of the subtransformational damage of C3H/10T1/2 cells. Experiments with low dose rates and in dependence of the state of proliferation.

III. Progress achieved:

III Progress acchieved

a) INDUCED TRANSPOSITION OF TY ELEMENTS IN YEAST:

1) Methodology: The quantification of Ty-transposition has been described in the previous reports. For the determination of the exact Ty-integration sites the sequencing method of Sanger has been used.

2) Results:

2.1) Transposition into chromosomal DNA: Three different types of mutations leading to an antimycin A resistant phenctype have been analysed: (i) Ty-transposition to the ADH2 locus; (ii) Ty-transposition to the ADH4 locus and (iii) Mutations without restriction fragment lenght polymorphism (RFLP) at both loci. In addition to spontaneously occurring mutants transposition is inducible by genotoxic agents: UV and gamma irradiation and the chemical agent ethyl methanesulfonate (EMS). Doses referring to equitoxicity for the different agents were chosen, resulting in 50%, 30%, 25% and 10% surviving fractions. In two haploid tester strains the overall mutation rate to antimycin A resistance was ten times higher than in the diploid strain resulting from these haploids. The fractions for the different mutant types, however, were equal in the three tester strains.

When the induction of transposition is compared at equitoxicity doses, EMS is the agent wich gives the highest fraction of insertional mutants. Gamma irradiation is more effective than UV irradiation. The transposition to the silent gene ADH4 is very frequent (appr. 20% of spontaneous up to 60% of induced mutants). Transposition to the ADH2 locus is normally less than 5% of the mutants. Only treatment with EMS gives Ty insertion 5' to the ADH2 structural gene that are up to 15% of the antimycin A resistant mutants. The EMS-induced transposition number will be lowered by inhibition of long-patch DNA synthesis with hydroxyurea (HU), by inhibition of translation with cycloheximide and by inhibition of the reverse transcriptase with azidothymidine (AZT). No effect was found by inhibiting transcription although first results indicate an increse of Ty-mRNA after mutagenic treatment.

2.2) Transposition into episomal DNA: Ty-integration sites were determined by DNA seqencing. Oligonucleotide primers were used homologous to highly conserved regions in the Ty-delta region. In this way not only the exact integration sites could be determined, but also the sequence of the Ty-delta could be compared. 4 of 4 Ty-delta insertions were found to have the same integration site and the same Ty-delta. From this we deduce that these mutants are identical and were isolated repeately. Also a solo-delta integration has been found at the ADH4 locus. In contrast to the literature it results in the same phenotype as complete Ty-integrations, i.e.: antimycin A

resistance due to overexpression of the gene. Among the antimycin A resistant mutants all Ty insertions at the ADH2 locus were localized 125 to 210 nucleotides upstream the ATG start codon. This area is flanked by the palindromic UAS (upstream activating sequence, the binding site for the activator) and a second palindromic sequence.

3) Discussion: All three genotoxic agents, gamma and UV-irradiation as well as EMS were found to induce Ty-transposition in haploid as well as in diploid strains. Although the mutant phenotype is dominant, the mutation rate in the haploid strains is tenfold higher than in the resulting diploid strain. Apparently that the normal cellular double-strand-break repair is not involved in Ty-transposition. Otherwise the transposition rate must have been higher in diploid than in haploid cells after gamma irradiation. This would explain, why ionizing radiation is far less effective than UV-irradiation. The qualitative analysis of antimycin A resistant mutants points to a "hot-spot" region of Ty integration sites at the ADH2 locus, while at the ADH4 locus the insertion sites are not so restricted to a certain position in the DNA sequence. This may be due either to the function of the 5' regions of the coding region (the ADH2 gene is expressed and regulated, the ADH4 gene is silent), or to the specificity of the Ty-integration system.

b) RADIATION INDUCED TRANSFORMATION IN MAMMALIAN CELLS:

1) Methodology: The system of the radiation induced transformation of C3H 10T1/2-cells was used as described.
2) Results and discussion: The experiments with low dose rate irradiation were continued. In previous studies we observed a definite decrease in transformation rate as dose-rate of gamma-rays was decreased from 0.1-1 Gy/min to 0.02 Gy/min and further, by another factor of two, by reducing the dose-rate to 2 Gy/d. The mechanism of this dose-rate effect was studied in delayed plating and in delayed irradiation and in split-dose experiments, altogether 8 large experiments comprising more than 3.000 dishes over the year. The transformation rate was not reduced significantly as the interval between plating and irradiation was increased to two days. Therefore, the very low dose-rate data cannot be explained by reduced sensitivity as was suggested in pilot experiments in 1987 but are due to slow repair processes. Delayed plating experiments showed significant repair of potentially transforming damage to occur but slower than potentially lethal damage repair. No subtransformation damage repair could be demonstrated in split-dose experiments. These studies on the effectiveness of protracted irradiation on cell transformation are approaching completion.

IV. Objectives for the next reporting period:

1. Quantitative analysis of Ty-mRNA after mutagenic treatment in haploid and diploid strains. 2.: Analysis of Ty-integration at the ADH2 locus after in-vitro deletion of the UAS-sequence. 3.: Construction of a recombinant Ty-element that can be overexpressed under defined conditions, then quantification of transposition after genotoxic treatment to look for the role of DNA-damage at the integration site.

2. The experiments about the transformation rate after exposure with low dose rates and about the underlying mechanism will be continued as described above.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. Dr. M. Ciriacy, Institut für Mikrobiologie der Universität, D-4000 Düsseldorf, F.R.C.

Prof. Dr. G.W. Barendsen, Radiobiological Institute, TNO, Rijswijk, Netherlands

Dr. V.M. Williamson, ARCO Plant Cell Inst. Dublin, Calif. U.S.A.

Dr. F. Eckardt-Schupp, Dr. F. Ahne, Institut für Strahlenbiologie der GSF, D-8042 Neuherberg

VI. Publications:

C. Morawetz, U. Hagen: Effect of irradiation and mutagenic chemicals on the generation of ADH2- and ADH4-constitutive mutants in yeast. 2. The inducibility of transposition by UV and ethyl methane sulfonate and the influence of metabolic inhibitors on the event. Mutation Research, in press.

W. Vogel: Entwicklung eines Systems zur Isolierung von Ty-Integrationen mit dem ADH2- und ADH4-Gen auf Plasmiden in Saccharomyces cerevisiae. - Charakterisierung der Integrationen. Dissertation, LMU München 1988/89.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Berkeley Nuclear Laboratories
Central Elect. Generating Board
Berkeley
GB - Gloucestershire GL13 9PB

Contract no.: BI6-D-095-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. T. Healey
Berkeley Nuclear Laboratories
Central Elect. Generating Board
Berkeley
GB - Gloucestershire GL13 9PB

Telephone number: 0453-810451

Title of the research contract:

Filtered neutron beam studies (biological effects).

List of projects:

1. Filtered neutron beam studies (biological effects).

Title of the project no.:

Filtered Neutron Beam Studies (biological effects)

Head(s) of project:

Dr A J Mill

Scientific staff:

Dr A J Mill
Ms S C Hall

I. Objectives of the project:

To undertake a wide-ranging study of the biological effects of intermediate-energy neutrons and assess implications for radiological protection, radiobiology and radiotherapy.

II. Objectives for the reporting period:

(i) To establish the biological effects of 10 keV and 24 keV filtered neutrons.

(ii) To investigate the relative biological effects of internally emitted low energy alpha particles.

1. Methodology

Two beams of intermediate-energy neutrons have been produced on heavy water moderated reactors at the UKAEA, Harwell Laboratory and used for RBE studies on cultured mammalian cells in vitro. A 24 keV neutron beam is obtained by using a filter of iron, aluminium and sulphur placed in a vertical thimble on the PLUTO reactor. The dose-rate in the beam during the first series of experiments was about 0.2 Gy/h with a gamma ray component of 0.03 Gy/h. In later experiments the neutron dose-rate was increased to about 0.34 Gy/h. 94% of the neutrons in the beam have an energy of 24 keV and these produce approximately 80% of the kerma in the beam. The diameter of the neutron beam is 5.3 cm.

A neutron beam with a mean energy of 10 keV is obtained using a filter of aluminium, sulphur and liquid argon, also in a vertical thimble, but on the DIDO reactor. Unlike the 24 keV beam, this beam is not truly monoenergetic. The neutron dose-rate is 64 mGy/h.

Two cell lines (HeLa S3 human cancer cells and C_3H 10T½ mouse fibroblasts) were used in the irradiations. The procedures for both HeLa and 10T½ cells were similar. Approximately six days prior to irradiation sufficient numbers of cells were inoculated into 35mm diameter petri dishes containing 1.5 cm^3 of growth medium and allowed to grow to confluence (in the case of HeLa) or near confluence (for 10T½). At this stage, the growth rates of the cultures are greatly reduced. This was necessary to suppress proliferation during the long irradiation times which, were up to 18 hours. The irradiations were carried out at 37°C, maintained by blowing air over the specimen. In order to prevent evaporation of the medium during exposure the dishes were sealed with thin plastic film.

Following removal from the neutron beam the medium was sucked off and trypsin added to detach the cells from the surface of the dish. The cells were then resuspended in about 5 cm^3 of growth medium and the cell concentration evaluated. The suspensions were diluted and replated onto fresh 10 cm diameter petri dishes and transported to Berkeley Nuclear Laboratories (BNL) in a battery-powered portable incubator. Here they were kept at 37°C for fourteen days prior to fixing and staining. HeLa cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 16% foetal calf serum (FCS), 1% non-essential amino acides and 2mM HEPES. For 10T½ cells the culture medium was Eagle's Basal Medium supplemented with 10% heat-inactivated FCS and maintained in an atmosphere containing 5% carbon dioxide. Survival for both cell lines was assessed on the usual criterion of fifty or more non-giant cells per colony.

Cells were irradiated either free-in-air (both 10T½ and HeLa) or at various depths within a polyethylene phantom (HeLa only). For the free-in-air exposures doses in the range 0.3 to 4.6 Gy of 24 keV neutrons were given. For the irradiations in a phantom, up to four dishes were placed at various depths within a 60 mm diameter polyethylene phantom and irradiated to total incident neutron fluences in the range 0.8 to 2.4 x 10^{12} cm^{-2}, corresponding to surface doses of between 2.3 and 6.5 Gy of

24 keV neutrons. With the 10 keV neutron beam, cells were exposed to a total incident neutron fluence of 1.4×10^{12} cm^{-2}, corresponding to a dose of 1.5 Gy.

In a separate series of experiments with HeLa cells boric acid, enriched in ^{10}B, was added to the medium to give final ^{10}B concentrations in the range 29μg/ml to 79μg/ml. Cells irradiated in the presence of such borated medium are additionally exposed to 1.5 MeV alpha particles (range in tissue about 9μm) produced by the reaction:

$$^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He + 2.79 \text{ MeV}.$$

Following irradiation, cells were removed from the dishes, resuspended, counted and plated. After incubation for 12 - 14 days dishes were washed in saline and fixed and stained with methylene blue. Colonies were scored on the usual criterion of 50 or more non-giant cells.

2. Results and Discussion

The survival curves obtained after irradiation with 24 keV neutrons are exponential with no evidence of a shoulder. This kind of survival curve is typically found for irradiation with densely-ionising radiation. Until these experiments were carried out it was not certain whether the low energy recoil protons produced by 24 keV neutrons would act like sparsely or densely-ionising radiation. These recoil protons have a maximum range of about 500 nm and the implication is that interactions between events greater than this do not contribute to cell death. The interpretation of data obtained with 24 keV neutrons using V79 cells is discussed in detail by Holt (1988).

The survival curves have been fitted to the data by a non-linear least squares regression analysis using the equation:

$$S = \exp(-\alpha D - -\beta D^2)$$

where S is the surviving fraction and D is the absorbed dose. For the 24 keV neutron curves, the value of β was taken as zero. The calculated values of α and β are given in Table 1, along with the low-dose rbe, calculated as the ratio of α values.

TABLE 1 Parameters of the best-fit lines calculated by least-squares regression for the relationship: $S = \exp(-\alpha D - \beta D^2)$ for HeLa and C_3H $10T\frac{1}{2}$ cells.

Cell line	250 kVp x-rays		24 keV neutrons	
	α/Gy^{-1}	β/Gy^{-2}	α/Gy^{-1}	rbe (ratio of α values)
HeLa S3	0.410 ± 0.059	0.045 ± 0.014	1.35 ± 0.04	3.3 ± 0.05
C_3H $10T\frac{1}{2}$	0.337 ± 0.035	0.007 ± 0.005	1.13 ± 0.04	3.4 ± 0.6

For cells irradiated in a phantom the additional cell-killing in borated cells is evident and a roughly proportional increase with boron concentration can be observed. The maximum boron effect is at a depth of about 2 cm where a ^{10}B concentration of 29μg/ml gives approximately thirty times increase in cell-killing over non-borated cells. For cells irradiated with 10 keV neutrons in a phantom the differential between non-borated and borated cells is enhanced even further.

Cells exposed to neutrons in the phantom are exposed to three different qualities of radiation. These are (i) alpha particles and lithium recoil nuclei (for borated cells only); (ii) protons from elastic scattering events with hydrogen and capture events in nitrogen and (iii) gamma ray photons incident in the beam and from capture events in hydrogen. In order to estimate the relative biological effectiveness of these components their contribution to the total dose at each depth must be estimated. Such calculations have been carried out using a Monte-Carlo technique to evaluate the spectrum of neutron energies at various depths within the phantom. The data set of 79 survival points were then analysed by multiple linear regression on the assumption that (i) the damage incurred from the three dose components was independent and (ii) the effects of each component was exponential. Thus:

$$\log_e(\text{survival}) = k_p D_p + k_c D_c + k_g D_g$$

where the subscripts p, c and g refer respectively to proton recoil, capture products and gamma ray components. D_{37} ($=-k^{-1}$) values of 0.84 ± .04 Gy for the recoil proton component, 0.78 ± 0.04 Gy for the neutron capture products and 10.3 ± 5.4 Gy for the gamma ray component were obtained.

Further experiments to investigate the transformation-rate of C_3H 10T½ cells after irradiation with 24 keV neturons are planned for the near future. Additionally, more data on the biological effects of 24 keV neutrons and their relevance to NCT are described by Morgan, Mill, Roberts, Newman and Holt (1988) and Mill and Harrison (1988).

3. Conclusions

The main conclusion to be made from these data is that the relative biological effectiveness of the recoil protons from 24 keV neutrons is high: approximately 3.3 compared with 250 kVp X-rays and approximately 1 compared with low energy alpha particles. These results have important implications for the quality factor used in radiological protection, for theories associated with radiation action and for the use of intermediate energy neutrons in neutron capture therapy.

REFERENCES

Mill, A.J. and Harrison, K.G., 1988. British Journal of Radiology, 61, 1147 - 1154.

Holt, P.D., 1988. British Journal of Radiology, 61, 1142 - 1146.

Morgan, G.R., Mill, A.J., Roberts, C.J., Newman, S.M., and Holt, P.D., 1988. British Journal of Radiology, 61, 1127 - 1135.

IV. Objectives for the next reporting period:

To extend the RBE experiments to other cell systems, in particular cell transformation in vitro in $C_3H/10T^1/_2$ mouse fibroblasts and micronucleus formation in human blood lymphocytes.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs G R Morgan, P D Holt and C J Roberts
Environmental and Medical Sciences Division
AERE Harwell
Oxon
OX11 ORA

Dr J W Hopewell
Research Institute
The Churchill Hospital
OXFORD
OX3 7LJ

VI. Publications:

Inactivation of Chinese Hamster V79/4(AH1) and HeLa cells by 24 keV neutrons.
Int. J. Radiat. Biol. 50, 35 - 40 (1986).

Biological Effects of 24 keV neutrons: RBE and Depth-Survival Data.
Proceedings of the 8th International Congress on Radiation Research, Edinburgh, July, 1987, 1, 314.

Boron Neutron Capture Therapy using Intermediate Energy Neutrons.
Proceedings of the 8th International Congress on Radiation Research, Edinburgh, July, 1987, 1, 316.

The radiobiology of 24 keV neutrons: measurement of the RBE free-in-air, survival and cytogenetic analysis of the biological effect at various depths in a polyethylene phantom and modification of the depth-dose profile by boron-10. British Journal of Radiology, 61, 1127 - 1135.

The interpretation of dose calculations and cell-survival measurements for the boron neutron-capture therapy of brain tumours with 24 keV neutrons. British Journal of Radiology, 61, 1147 - 1154.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Rue Charles Lemaire, 1
B - 1160 Bruxelles

Contract no.: BI6-D-090-B

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M. Janowski
Département de Radiobiologie
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Telephone number: 014-31.18.01

Title of the research contract:

Mechanism of radiation-induced leukemogenesis and osteosarcomagenesis.

List of projects:

1. Mechanisms of radiation-induced leukemogenesis.
2. Molecular biology of radiation-induced osteosarcomagenesis. Role of oncogenes and viruses.

Title of the project no.: 1

Mechanisms of radiation-induced leukemogenesis

Head(s) of project:

M. Janowski

Scientific staff:

M. Janowski, B. Borremans, R. Hooghe

I. Objectives of the project:

For three decades, retroviruses have been suspected - although not demonstrated unequivocally - to play a role in radiation-induced leukemogenesis in mice. Radiation leukemia virus is a good tool to investigate the molecular mechanisms by which it exerts its effects and to trace back the events leading to the appearance and development of thymic lymphomas upon irradiation.

II. Objectives for the reporting period:

Objective 1: To extend our previous observations that novel recombinant retroviral genomes occur in the DNA of radiation-induced thymic lymphomas. To test for the hypothesis that they might be integrated in a critical region. To attempts to define that region, which might be an oncogene.
Objective 2: To identify factors other than retroviruses, that might contribute to the malignant transformation, such as mitogenic growth factors.

III. Progress achieved:

1. Methodology

The fact that nonirradiated thymic grafts develop into thymic lymphomas in a thymectomized irradiated host shows that the irradiation activates a diffusible leukemogenic factor, capable of provoking the malignant transformation of the grafted cells. To assess the donor origin of the tumors, mice bearing the Thy-1.2 membrane antigen were thymectomized, submitted to 4 weekly X ray doses of 1.75 Gy, and grafted with thymuses from congenic newborn mice bearing the Thy-1.1 allotype.

In the frame of objective 1, classical techniques of molecular biology were used throughout to analyse the DNA and the RNA of both classically and indirectly induced tumors and tumor cell lines. An important molecular probe used in molecular hybridization experiments was pEc-B4, which very specifically recognises the unique endogenous ecotropic provirus, and not the many endogenous xenotropic proviruses of the C57BL/Ka mouse strain

In the frame of objective 2, the possible involvement of IL-1 and IL-6 was studied by the inhibition of their synthesis with indomethacin, present as a 10 µg/ml solution in the drinking water from the 8th day before the first irradiation. The possible role of IL-2 was investigated by injecting three times a week the immunosuppressive peptide cyclosporin A (25 to 100 mg/kg) from the day before the first irradiation. In situ molecular hybridization was used to detect the expression of specific RNAs.

2. Results.

Objective 1.

We have already reported that, in the rat, 90% of radiation leukemia virus-induced thymic lymphomas expressed a 2.4 kb polyadenylated RNA, synthesized from a proviral promotor but nevertheless distinguishable from the classical 8.3 and 3.4 kb viral RNAs. This RNA was identified as a viral, envelope-specific RNA, from which sequences were deleted due to the use of an alternative splicing site during its processing.

Using the pEc-B4 probe, we searched for the presence of novel ecotropic recombinant proviruses, both in tumors induced by the classical irradiation scheme (4 x 1.75 Gy) and in tumors that arose from nonirradiated thymuses that were grafted into thymectomized, irradiated recipients. Either leukemogenic protocol yielded similar results: in 30 % of the tumors analysed, novel proviral sequences were detected, presenting hybrid properties between the endogenous ecotropic (hybridisation with pEc-B4) and xenotropic (a specific recognition site for the restriction enzyme KpnI). The proviral integration patterns were most of the time clonal, as attested to by the use of restriction enzymes (EcoRI and HindIII) cleaving the cellular DNA in the sequences flanking the provirus. Moreover, many of the tumors that did not display a novel provirus became positive in this respect after in vivo transplantation or in vitro cultivation. Thus, the proviruses had to be present in the initial tumor, although in a too small proportion of the cells to be detectable. Moreover, these cells had acquired a selective growth advantage, allowing them to undergo clonal growth as transplants or cultures. Our colleagues of the INSERM Unit n° 117 in Bordeaux cloned the sequences flanking one of the proviruses and identified them as the presumed oncogene Mlvi-1. However, none of the other tumors investigated showed a proviral insertion in this domain.

Objective 2.

In situ hybridizations revealed no increased synthesis of IL-1 or IL-6 RNA in radiation-induced thymic lymphomas as compared to normal thymuses. Mice in which the production of IL-1 and IL-6 was inhibited with indomethacin developed radiation-induced thymic lymphomas with the same yield and the same latency as control mice.

Continuous treatment with cyclosporin A had no influence on the yield, the latency and the biological characteristics of the tumors. Cell lines were established from lymphomas appearing during cyclosporin A treatment. Their membrane phenotype was similar to that of lines established from mice that had been irradiated without any additional treatment.

Recently, we obtained evidence that the insulin-like growth factor II was expressed at higher levels in radiation-induced tumors than in control thymuses.

3. Discussion

Objective 1.

Altogether, and due to the use of a very adequate molecular probe, the data from our collaborative effort with INSERM Unit n° 117 bring for the first time evidence of integration of novel ecotropic recombinant proviruses in a nonnegligible proportion (30 %) of primary, radiation-induced thymic lymphomas of the mouse. This proportion is even significantly higher when one considers that many of the tumors contain novel integrated proviruses in a limited number of cells, as revealed after transplantation or cultivation. The latter phenomenon emphasises the growth advantage acquired by these cells due to the presence of a provirus. However, the role of the provirus might be limited to tumor growth rather than to the initiation process. Therefor, it should be investigated if the initial event could be due to another mechanism, such as a point mutation in a member of the ras gene family, as reported in the literature for a variety of human and animal tumors, and suggested formerly by preliminar experiments on radiation-induced thymic lymphomas of C57BL mice.

Objective 2.

We did not find evidence that the growth factors IL-1, IL-2 or IL-6 play a role in radiation induced thymic lymphomagenesis. However, the insulin-like growth factor II deserves further investigation, in view of its elevated expression in the tumors.

IV. Objectives for the next reporting period:

Objective 1.

The possible contribution of insulin-like growth factors will be investigated at the mRNA and at the protein level.

Objective 2.

Molecular cloning of the cellular DNA sequences flanking the novel provirus in a primary radiation-induced tumor, in order to probe other primary tumors for a possible more general involvement of this domain.

Screening primary radiation-induced tumors for mutations in the ras genes, taking advantage of the specific gene amplification technique with the polymerase chain reaction.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Unité INSERM n° 117, Fondation Bergonié, 229 Cours de l'Argonne, F-33076 Bordeaux, France.

VI. Publications:

R. Hooghe, E. Hooghe-Peters & J.Van Snick: Interleukin-6 production in murine T-cell lymphomas. Ann.N.Y.Acad.Sci., in press.

H.Baylac-Kalabokias, T.Astier-Gin, B.Borremans, E.Legrand, R.Hooghe, M.P.Houben-Defresne, M.Janowski, J.F.Duplan & B.Guillemain: Evidence of recombinant ecotropic provirus in thymic lymphomas induced by direct or indirect radiation effects. Leukemia Res., in press.

R.Hooghe, M.Janowski, R.Greimers & N.Schaaf-Lafontaine: Radiation-induced lymphomas developping during Cyclosporine A treatment. Submitted.

Abstracts:

M.Janowski, B.Borremans, R.Hooghe, J.Boniver & M.P.Defresne: The mechanisms of action of radiation leukemia virus and its relevance to radiation-induced leukemogenesis in mice: Symposium on the Molecular and Cellular Mechanisms of Biological Radiation Effects, Munich, 23-25 mars 1988.

M.Janowski, H.Baylac-Kalabokias, T.Astier-Gin, B.Borremans, E.Legrand, R.Hooghe, M.P.Houben-Defresne, J.F.Duplan & B.Guillemain: Radiation-induced thymic lymphomagenesis: Discussion of the hypothetical viral etiology. Proc. Proc. 21st Annual Meeting of the European Society for Radiation Biology, Tel Aviv (Israel), 24-30 octobre 1988.

M.Janowski: Symposium on the Molecular and Cellular Mechanisms of Biological Radiation Effects, Munich, 23-25 mars 1988: The mechanisms of action of radiation leukemia virus and its relevance to radiation-induced leukemogenesis in mice.

M.Janowski: EULEP Workshop on Radiation-induced Leukemias and Osteosarcomas, Bordeaux (France), 29-30 mars 1988: The molecular mechanisms of radiation-induced thymic lymphomagenesis.

M.Janowski: 21st Annual Meeting of the European Society for Radiation Biology, Tel Aviv (Israel), 24-30 octobre 1988: Radiation-induced thymic lymphomagenesis: Discussion of the hypothetical viral etiology.

Title of the project no.: 2

Molecular biology of radiation-induced osteosarcomagenesis. Role of oncogenes and viruses.

Head(s) of project:

B. Borremans

Scientific staff:

B. Borremans
M. Janowski

I. Objectives of the project:

To identify and characterise genes submitted to critical alterations in relation with radiation induced osteosarcomagenesis.

Searching for reorganisation of proto-oncogenes leading to their activation.

II. Objectives for the reporting period:

III. Progress achieved:

1. Methodology

Classical restriction enzyme analysis of DNA with specific probes for molecular hybridization.

2. Results

In the frame of project n° 1, we obtained indications that the presumed proto-oncogene Mlvi-1 seems sometimes to be involved in radiation leukemia virus-induced leukemogenesis of rats. On the other hand, our colleagues of the INSERM Unit n° 117 (Bordeaux, France) performed the molecular cloning of a gene that could be rearranged in radiation-induced leukemogenesis of C57BL mice. Our colleagues of the GSF (Neuherberg, FRG) provided us with a series of DNAs from radiation-induced osteosarcomas of Balb/c mice. We tested these DNAs for rearrangements of the gene that was cloned in Bordeaux, and found that it was frequently and considerably amplified. It turned out that this amplifications occurred in the tumors that already had been shown to display concommitant amplification of the proto-oncogene c-myc and of the presumed oncogene Mlvi-1. This result contributed to the identification of the gene studied in Bordeaux as Mlvi-1, and simultaneously confirmed the observations made in Neuherberg.

3. Discussion.

Radiation-induced osteosarcomas of the Balb/c mouse frequently display concommitant amplification of the proto-oncogene c-myc and of the presumed proto-oncogene Mlvi-1. This phenomenon is due to the appearance of multiple minute chromosomes containing both c-myc and Mlvi-1. However, the contribution of these minute chromosomes to the osteosarcomagenetic process is still unknown.

IV. Objectives for the next reporting period:

The molecular biology studies were performed on transplanted, and not on primary osteosarcomas. It should be investigated if the gene amplifications also can be observed in the primary tumors.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Abteilung für Pathologie, Gesellschaft für Strahlen- und Umweltforschung, München, D-8042 Neuherberg, FRG.

Department of Molecular Biology, University of Aarhus, Möllers Allé 130, DK-8000 Aarhus, Denmark.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

University of Aarhus
Ndr. Ringgade 1
DK - 8000 Aarhus C

Contract no.: BI6-D-086-DK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. N.O. Kjeldgaard
Dept. Molecular Biol. & Plant Phy.
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DK - 8000 Aarhus C

Telephone number: 06-125177

Title of the research contract:

Characterization of somatic mutations during radiation induced osteosarcomagenesis.

List of projects:

1. Characterization of somatic mutations during radiation induced osteosarcomagenesis.

Title of the project no.: [1]

Characterization of somatic mutations during radiation induced osteosarcomagenesis.

Head(s) of project:

N. O. Kjeldgaard and F. S. Pedersen

Scientific staff:

H. Y. Dai, E. Jørgensen, P. Jørgensen, N. O. Kjeldgaard, J. Lovmand, S. Lovmand, H. S. Olsen, N. Pallisgaard, K. Paludan, F. S. Pedersen, L. Pedersen.

I. Objectives of the project:

1) To identify genetic changes in proviral genes and other cellular ganes associated with integrated proviruses occuring during the development of bone tumours.

2) To determine the functional role of these mutations and to evaluate their role in osteosarcomagenesis.

II. Objectives for the reporting period:

1) To isolate and characterize additional viruses from bone tumours.

2) To perform detailled transcriptional analysis of the isolated viruses.

3) To analyze the cellular genes surrounding integrated proviruses in bone tumours.

III. Progress achieved:

All our work employs murine retroviruses as tools for the study of radiation induced carcinogenesis.

1) Isolation and characterization of viruses from bone tumours.
All viruses included in this analysis are handled as molecular clones in bacterial vectors. Viral isolates were obtained from radiation induced osteosarcomas of BALB/c mice (Strauss et al. 1988 and unpublished results). These viruses have, together with other available BALB/c viruses of related structure, been included in our studies of viral structure and pathogenicity. Partial nucleotide sequence analysis of the viruses indicated only minor differences, whereas marked differences were observed in the pathogenicity of the viruses in NMRI mice. The osteosarcoma derived viruses showed little or no induction of osteopetrosis, in contrast to a clear induction caused by the endogenous ecotropic BALB/c virus and by an exogenous derivative. Identification of the structural features of the viral genomes determining these biological differences may reveal molecular aspects of viral effects on bone tissues. Studies of structural determinants of viral pathogenicity are also carried out for osteoma inducing viruses among which the bone tumour derived RFB MuLV and OA-I MuLV are the most potent isolates and for T-lymphoma inducing viruses using SL3-3 muLV as the prototype.

2) Analysis of the transcriptional properties of the viruses.
These studies are based on the finding of variation in arrangement of nucleotide sequences regulating viral transcription and on the confirmed role of the LTR for the oncogenic properties of some murine leukemia viruses.
The vectors used for our gene transfer experiments have been described in previous reports.

In one type of experiment DNA containing an LTR controlled transcription unit is transferred to cells in culture and the LTR driven expression is measured after about two days. As recepient cells we have used NIH fibroblasts for the initial studies, but osteogenic cells have now also been included. In the assays the LTRs are used either directly or after modification by deletion or site-specific mutagenesis. Using this approach we have extended our previous studies of the Akv LTR and of the natural LTR variants from OA-I and SL3-2. The LTR of another bone tumor associated virus, RFB, has also been included in recent studies.

Our studies of host protein factors that may determine the function of the critical LTR regions as defined by the expression studies have been continued. The studies of DNA binding proteins have focused upon Nuclear Factor I, a protein factor of general significance for murine leukemia virus expression and upon a protein factor showing differences in binding to the sequences of the Akv and OA-I

LTRs. Using a somewhat different approach, the transcriptional activity of an LTR in the cells is determined after co-transfer with a gene coding for a protein with a possible influence on LTR activity. Our present experiments of this sort investigate the effect of the bone tumour derived oncogene, fos, upon LTR activity in fibroblasts and in osteogenic cells.
To study the role of the nucleotide sequence differences in the LTR for the transcriptional activity of integrated proviruses our retroviral neo-transmission vectors are employed. The defectiveness of these recombinant viruses allow for only one replication cycle. In initial experiments the lymphoid cell line L691 was infected by vectors carrying the neo gene under control of either the Akv LTR or the lymphotropic SL3-3 LTR. For the infected cell populations the transcriptional activity of the SL3-3 LTR was only about twice that of the Akv LTR, in contrast to the difference of about one order of magnitude observed for unintegrated DNA after DNA-mediated gene transfer. The transcriptional activity of the infected cells, however, showed large variation between individual proviruses, presumably due to an influence from the site of integration. Thus, the marked difference in oncogenicity between the SL3-3 LTR and the Akv LTR therefore seems to be associated with a relatively small difference in their effect on the average virus expression from an infected cell population. The oncogenic function of the LTR may therefore be mediated in other ways, possibly through an effect upon expression of other genes in the vicinity of the provirus. These results bear general relevance to studies of the effect of DNA rearrangements upon gene expression.

3) Analysis of cellular genes surrounding integrated proviruses in bone tumours.
Analyses of provirus integration patterns have been performed for radiation induced osteosarcomas (Strauss et al.,1988) For these studies many types of endogenous retroviral-like elements may be potentially relevant as insertional mutagens. Benign bone-tumours (osteomas) induced by defined viruses may however also serve as a model system to define host gene targets of possible relevance for radiation induced osteosarcomas. Extraction of the DNA from osteomas is complicated by their small size of these tumours and by their calcification. One way to circumvent these problems would be to reduce the quantities of DNA required for the analysis. The suppressor tRNA (supF) provirus tagging (P. Jørgensen et al. 1988) was developed to simplify the analysis of the host DNA flanking integrated proviruses. In collaboration with GSF/Neuherberg we have found that a supF tagged SL3-3 virus retains its lymphomagenic properties and that the supF containing proviruses can be recovered from the tumour cells. For this system the analysis of flanking DNA regions is in progress. Another approach that we have taken consists in using a variant of the polymerase chain reaction (PCR) technique for selective DNA amplification of provirus-host junctions. Combinations of these methods will be used to analyse the DNA surrounding integrated proviruses in bone tumours.

IV. Objectives for the next reporting period:

1) To identify genes involved in bone tumour formation by
   a) locating determinants for pathogenic specificity on the genome of retroviruses causing bone disease
   b) analyzing cellular genes surrounding integrated proviruses in bone tumours

2) To study virus bone cell interaction at the level of regulation virus gene expression.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

The work is performed in close collaboration with the research groups at GSF/Neuherberg (Erfle, Gössner) and Mol (Janowski) under the coordination of EULEP Task Group no. 1 on "Radiation induced osteosarcomagenesis" and Task Group no. 2 on "Radiation induced lymphomagenesis".

VI. Publications:

Jørgensen,P., Mikkelsen,T., Pedersen,F.S. & Kjeldgaard,N.O.: A MuLV transmission vector system designed to permit recovery in E.coli of proviral and cellular flanking sequences. Virus Genes 1, 221-233, (1988).

Strauss,P.G., Schmidt,J., Pedersen,L., & Erfle,V.: Amplification of endogenous proviral MuLV sequences in radiation-induced osteosarcomas. Int.J.Cancer, 41, 616-621 (1988).

Copeland,N.G., Jenkins,N.A., Nexø,B., Schultz,A.M., Rein,A., Mikkelsen,T., & Jørgensen,P.: Poorly expressed endogenous ecotropic provirus of DBA/2 mice encodes a mutant Pr65gag protein that is not myristylated.J.Virol. 62, 479-487, (1988).

Jørgensen,E.C., Kjeldgaard,N.O., Pedersen,F.S., & Jørgensen, P.: A nucleotide substitution in the gag N terminus of the endogenous DBA/2 virus prevents Pr65-gag myristylation and virus replication. J. Virol. 62, 3217-3223, (1988).

Kjeldgaard,N.O., Bækgaard,A.J., Dai,H.Y., Etzerodt,M., Jørgensen,P., Lovmand,S., Olsen,H.S., & Pedersen,F.S.: Transcriptional control by retroviral LTR regions. In Evolutionary Tinkering in Gene Expression. NATO Advanced Summer Institute Series (M. Grunberg-Manago ed.), Plenum Publishing Corp., New York, USA, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Rijksuniversiteit Leiden
Stationsweg, 46
NL - 2300 RA Leiden

Contract no.: BI6-D-202-NL

Head(s) of research team(s) [name(s) and address(es)]:

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Rijksuniversiteit Leiden
Wassenaarseweg, 72
NL - 2333 AL Leiden

Telephone number: 071-14833 Ext. 6176/6150

Title of the research contract:

Genetic and molecular characterization of stages in X-ray induced malignant transformation.

List of projects:

1. Investigation of the number and the genetic and molecular nature of the events, such as immortalization, transformation, oncogene activation, promotion, which are involved in radiation induced carcinogenesis using Syrian hamster embryo cells.

Title of the project no.: 1

Investigation into the number and the genetic and molecular nature of the events, such as immortalization, transformation, oncogene activation, promotion, which are involved in radiation induced carcinogenesis using Syrian hamster embryo cells.

Head(s) of project: Dr. J.W.I.M. Simons

Scientific staff:
Drs. A.J. de Kok
Drs. B. Bols

I. Objectives of the project:

This project aims to further define the events in malignant transformation of cultured cells. These events are immortalization, transformation and promotion. The search will be for genetic mechanisms and genes involved.

II. Objectives for the reporting period:

-Induction of immortality by X-rays.
-Study on complementation analysis in immortality via cell fusion.
-Expression of proto-oncogenes in successive stages of transformation of SHE cells.

III. Progress achieved:

Induction of immortality in SHE cells.

Methodology

Tertiairy SHE cells have been treated with B(a)P (20 ug/ml) or with X-rays (300 rad). The treated cells were split into a large number of subpopulations and cultured for 6 days in complete medium for expression of induced mutations and recovery from the treatment. Subsequently each population was cultured in medium with low serum in order to select for immortalized cells. The cultures were propagated until senescense or immortalization was observed. Immortalization frequencies were determined with the P-zero method on the proportion of cultures which escaped normal senescense.

Results

Spontaneous immortalization has not been observed. Pooling of the data from the controls therefore indicates that the frequency of spontaneous immortalization must be below $3.7X10^{-6}$ per cell per generation.
Immortalization has been induced both by B(a)P and X-rays. The frequencies of immortalization were $2.2X10^{-4}$ and $2.4X10^{-5}$ respectively.

Discussion

The induced immortalization frequencies are comparable to frequencies of induced mutations in one allele. The absence of spontaneous immortalization does however not fit with the explanation that one mutation is sufficient to start immortalization in SHE cells. Therefore it has to be investigated whether immortalization has been induced directly after treatment or whether a two-step mechanism (activation followed by rare alterations) is involved as has been suggested for X-ray induced cell transformation.
The data show that the cells which have been induced to escape normal senescense stil have to undergo more changes in order to become fully immortalized. One is enhancement of the cloning efficiency and another fast growth rate. Both events appear to take place at random and spontaneously. This requires further investigation.

Study on complementation in immortality via cell fusion.

Methodology

A TOR-mutant (resistant to thioguanine and ouabain) of an immortal cell line was fused with itself, with wild type SHE cells and with 8 other immortal cell lines. From each cross 25-40 proliferating hybrids were isolated and tested for lifespan.
In another series of experiments cells were transfected with plasmids with either the gene for neomycin resistance or hygromycin resistance in order to perform the selection of hybrids with two dominant markers.

Results

Fusion of the immortal line with itself led for 90% to immortal hybrids; fusion of the immortal line with mortal cells produced 75% mortal hybrids and fusion of the immortal line with 8 other immortal lines produced a range of 25% to 100% of immortal hybrids.
Experiments on transfection of the gene for neomycin resistance produced G418 resistant cells, but induced also cell transformation of wild type SHE cells.

Discussion

Although evidence was obtained for complementation and absence of complementation in the fusions with wild type cells and immortal cells respectively the results are unsatisfactory because clearcut data on complementation groups will be difficult to obtain. The reasons for this are sought in the long procedure for obtaining TOR-mutants which may alter the characteristics of the cell line and in a frequent arising of variants

within the hybrid populations.
As transfection of plasmids with dominant markers induced a high frequency of cell transformation, this approach is not promising either. A solution to the problem is now sought in the transduction of dominant markers with the aid of retroviral vectors.

Expression of proto-oncogenes in successive stages of transformation of SHE cells.

Methodology

Normal, immortal (BP-A), ENU-transformed BP-A and BP-A cells transformed by the pEJ-cHa-ras oncogene were compared in the expression of five cellular proto-oncogenes (myc, p53, c-H-ras, erb-B, c-sis) to investigate whether sequential steps in malignant transformation are characterized by a pattern of oncogene activation.

Results

The level of c-myc transcripts was similar in normal, senescent, immortal or transformed cells and did not decrease in cells incubated in serum-free medium. The expression of p53 did increase in transformed cells, but this expression correlated with the expression of histon genes. The level of c-Ha-ras transcripts was similar in all stages of neoplastic conversion, except in the pEJ-induced transformant in which it was significantly enhanced. No erbB transcripts were detected and expression of c-sis was not observed in normal, immortal and pEJ-induced transformants. In 1 out of 9 ENU-induced transformants expression of c-sis was detected.
Finally, southern analysis revealed no rearrangements in the five genes studied.

Discussion

C-myc and p53 were examined as it has been shown that they can play a role in immortalization; c-Ha-ras is known to be involved in transformation and c-sis and c-erbB were chosen because they code for a growth factor and growth factor receptor respectively and thus could be involved in the observed loss of growth factor requirment of the transformed cells.
The results indicate that c-myc is not involved in the immortalization or transformation of SHE cells. As the increase in p53 transcription correlates with transcription of histon genes it is most likely that this increase is largely due to the increase in number of cycling cells. It is also concluded that c-Ha-ras, c-erbB and c-sis do not play a predominant role in immortalization or transformation of SHE cells.

IV. Objectives for the next reporting period:

A) analysis of the number of steps involved in immortalization of SHE cells. a) determination of frequency per cell per generation of 1) increase in cloning efficiency and 2) increase in growth rate; are these steps independent. b) investigation on the question whether carcinogenic treatment induces directly extension of the lifespan or indirectly via an activation mechanism, followed by rare alteration in the progeny of the treated cells. B) karyotype analysis of cells in the different stages of immortalization. C) introduction of the genes for neomycin resistance and hygromycin resistance with retroviral vectors. Application of these dominant markers for cell fusion. D) Effect of the tumor promotor TPA on the frequency of immortalization of SHE cells. E) Effect of the tumor promotor TPA on genetic stability.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Department of Molecular carcinogenesis, University of Leiden. (Prof. Dr. A.J. van der Eb).

VI. Publications:

Kok, A.J. de, H.M. Sips, L. den Engelse and J.W.I.M. Simons. Epidermal growth factor enhances N-ethyl-N-nitrosourea induced morphological transformation of Syrian hamster embryo cells. Carcinogenesis 9 661-664 1988

Kok, A.J. de, H. Sips, L. den Engelse and J.W.I.M. Simons. Prolonged in vitro exposure of Syrian hamster embryo cells to 3-aminobenzamide induces transformation and chromosomal alterations but not gene mutations. Carcinogenesis (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Federated Dublin
Voluntary Hospitals
P.O. Box 795
IRL - Dublin 8

Contract no.: BI6-D-093-IRL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.F. Malone
Dept. Med. Physics & Bioengineering
P.O. Box 580
St. James's Hospital
IRL - Dublin 8

Telephone number: 01-537941 ext. 2648

Title of the research contract:

Radiation response of the thyroid : survival and alteration towards malignancy in cell culture and human systems.

List of projects:

1. Studies of radiation induced effects on thyroid cell survival and function, and of the dosimetry of radioiodine in cell cultures.
2. Carcinogenic aspects of thyroid irradiation.

Title of the project no.: 1

Studies of Radiation Effects on Thyroid Cell Survival and Function, and on the Dosimetry of Radioiodine in Cell Culture.

Head(s) of project: J. F. Malone.

Scientific staff: J. F. Malone,
N. Sheahan,
M. Lewis,
B. Tuohy.

I. Objectives of the project:

This project has two objectives. First to study the biological responses (other than neoplastic development) to irradiation; and secondly to attend to the necessary developments in dosimetry.
The first study will, in particular, concentrate on the survival, recovery and function of thyroid cell cultures after irradiation. In addition alterations in cell function will be monitored. These studies will contribute to the understanding of non-malignant radiation induced thyroid diseases, as well as provide correlative information for the carcinogenic studies in Project 2, as both sets of gross endpoints are strongly interrelated. Finally, they will indicate if the status of the thyroid in vivo might be manipulated to prevent undesirable radiation sequelae.
The dosimetric studies involve detailed micro dosimetric monitoring of the structure of thyroid cell culture using a sophisticated computer model and image analysis computer, as well as microscopic and macroscopic experimental investigations to confirm these results.

II. Objectives for the reporting period:

(1) To continue the study of survival recovery and functional studies, with emphasis on comparison of 60-Co and 131-I data.

(2) To continue microdosimetric studies of radioiodine in the thyroid.

(3) To extend the studies of the relative contributions to survival of the epithelial and fibroblast in culture, and the identification of a stem cell polulation, should one exist.

(4) To continue immunocytochemical and autoradiographic studies prior to and after irradiation.

(5) The continued development of a unified cell kinetics/radiation response model to account for the observed post-irradiation behaviour of the thyroid, the progress report for which may be found under Project 2.

III. Progress achieved:

Much progress has been achieved in 1988 with the clonogenic survival assay for cells of ovine origin, particularly in the comparison of 60-Co and 131-I data, and the relative contribution of colonies of epithelial and fibroblast origin to the total survival.

METHODOLOGY: The tissue culture methodology is the same as that previously used. The clonogenic assay has been used for examination of 60-Co and 131-I cell survival and differential counts of colonies of epithelial and fibroblast origin undertaken. An experiment, previously designed to test the degree to which the higher Do value for 131-I is due to dose rate, has been completed. This involved multifractionation of an absorbed dose of 14Gy of 60-Co irradiation over a 7 day period with the number of fractions ranging from one per week to two per day for 7 days.

RESULTS AND DISCUSSION:

1. Further experiments carried out using acute doses of 60-Co gamma radiation on cells of ovine origin have resulted in Do values ranging from 1.6-2Gy, on consideration of epithelial colonies only. The extrapolation numbers obtained are higher than those previously reported, and are in the range 13-19. The observed shoulder indicates an expected recovery within several hours of irradiation. This recovery was observed 3-6 hours after irradiation, with recovery curves showing a recovery factor in the range of 3-5, which correlates with previous work. A split dose survival curve experiment was also carried out, with a conditioning dose of 10Gy. This also showed recovery with a Do value of 3.8Gy. (See Figure 1).

2. Considering colonies of fibroblast origin in the total surviving fraction of single dose survival curves, after 60-Co irradiation, had little or no effect on the Do values obtained, but did result in a notable alteration of shoulder size, with extrapolation numbers ranging from 17-21. However during split dose experiments no significant effect on either Do values or extrapolation numbers was observed on inclusion of these colonies in the total survival level.
Table 1 shows the mean level of fibroblast colonies obtained during single dose survival experiments, for 60-Co irradiated sheep thyroid cells, expressed as a percentage of the total colonies counted.

Table 1: Percentage of fibroblast colonies obtained on irradiation of sheep thyroid cells with acute doses of 60-Co.

Dose:	0	1.5	2.5	5	7.5	10	12.5	15
% Fibroblast Colonies:	11.45	12.9	10.9	13.9	14.7	19.3	19.1	19.9

3. An experiment involving multifractionation of a 14Gy dose of 60-Co has been completed and the expected increase in survival with increasing number of fractions was observed, with little increased recovery between 7 and 14 fractions per week. A comparison of this data with previous 131-I survival experiments showed that with larger numbers of fractions the survival values were greater than those for 131-I. These results seem to agree with previous results for 60-Co/131-I comparisons in which the ratio of 60-Co:131-I was determined to be of the order of 5, and indicate that dose rate alone could account for the 131-I effect (See Figure 2).

3. Similar experiments using fractionated doses of 60-Co are planned for cell cultures of human origin, where the 60-Co:131-I ratio was previously determined to be of the order of 6.

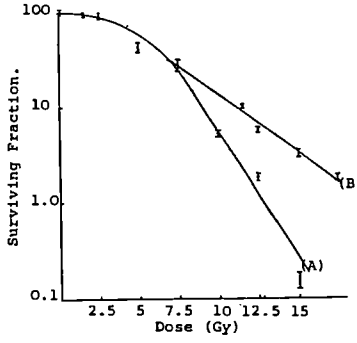

Fig. 1.

(A) Single dose survival curve and

(B) Split dose survival curve (with Dc=10Gy) for sheep thyroid cells irradiated with 60-Co (epithelial cells only).

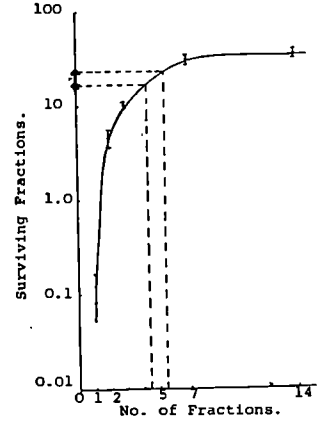

Fig. 2.

I-surviving fraction (epithelial sheep thyroid cells versus no of fractions of 14Gy dose of 60-Co.

I-range of survival over 6 experiments of sheep thyroid epithelial cells irradiated with 131-I.

IV. Objectives for the next reporting period:

(1) To complete the study of survival, recovery and functional studies of cells of ovine and human origin, with emphasis on comparison of 60-Co and 131-I data.

(2) To extend the studies of relative contribution to survival of the epithelial and fibroblast in culture, and the identification of a stem cell population, should one exist.

(3) To continue the application of immunocytochemical and autoradiographic methods in attempting to identify proliferating units, differentiated units, and after irradiation.

(4) To complete microdosimetric studies of radioiodine, with emphasis on the clarification of the gamma component of dose.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. C. Seymour et al., Radiobiology Laboratory, St. Luke's Hospital, Highfield Road, Rathgar, Dublin 6.

Prof. M. Cullen, St. James's Hospital.

Prof. J.E. Dumont, Hospital Erasme, Brussels.

VI. Publications:

See Project 2.

Title of the project no.: 2

Carcinogenic Aspects of Thyroid Irradiation.

Head(s) of project: J.F. Malone.

Scientific staff: J.F. Malone,
N. Sheahan,
B. Tuohy,
P. Gilligan.

I. Objectives of the project:

To determine and quantify the carcinogenic effect of acute and radioiodine irradiation to the thyroid using differentiated cell culture transformation models already developed. Particular efforts will be made to do the work on human material. To attempt to quantify the dose response relationship with respect to the induction of specific features of transformation in thyroid cells. To determine the contribution of 131-I iodine taken up by follicles on the rate of transformation induction. To explore the low dose and low dose rate effects and determine a reliable "r.b.e.' value for 131-I. Finally to continue the epidemiological study of radiation carcinogenesis in the patient follow-up studies being co-ordinated through this programme.

II. Objectives for the reporting period:

1. To continue to quantify the dose-response relationship.
2. To attempt to confirm the apparent absence of transformation following 131-I irradiation, and to investigate the mechanism underlying this observation.
3. To continue attempts to culture small amounts of human thyroid material which has been subject to irradiation 'in-vivo'.
4. To develop a unified cell kinetics/radiation response model to account for the observed post-irradiation behaviour of the thyroid.

III. Progress achieved:

METHODOLOGY:

The tissue culture methodology previously described has been used in the culture of both human and sheep thyroid tissue. The assays for transformation frequency developed over the years are being used in epithelial cell transformation studies. The soft agar growth assay appears to be a reliable and reproducible assay for transformation. The development of focus formation and LDH isoenzyme analysis as transformation assays is continuing and their validity being assessed.

RESULTS AND DISCUSSION:

1. A total of 10 sheep thyroids have been cultured and the confluent differentiated monolayers exposed to 131-I for 7 days, with a dose range of 10-15Gy. At the end of the exposure period the survival of control cultures was determined using the clonogenic technique. Cells from the same cultures were plated, grown to confluence and the plating efficiencies again determined. This process was repeated until control cells had senesced, (passage 4 for sheep thyroid cells). At this point any irradiated cells which survive are potentially transformed as they have undergone the first step (delayed senesence) characteristic of transformed cells. Surviving cells were tested for growth in soft agar at each subculture from this point on 131-I treated sheep cells senesced at the 2nd subculture and hence there is an apparent lack of transformation as assessed by this method. However as with most negative scientific results, caution in accepting this conclusion is warrented, and we intend persisting further with these investigations to ensure methodology problems are not present.

A complex longterm experiment designed to determine whether this lack of transformation in 131-I irradiated sheep thyroid cells reflects the real properties of 131-I or some abberrant behaviour, is underway. It is hoped that on completion this will yield some definite results.

2. Transformation using the soft agar endpoint has not been detected in human thyroid cultures after 60-Co gamma irradiation and investigations to determine whether this is due to the need for technical modifications of this technique (which yields positive results for 60-Co gamma irradiated sheep thyroid cultures) or to the real properties of human thyroid tissue are continuing. Investigations into the lack of 131-I induced transformation in human tissue are continuing along the same lines as those for sheep thyroid tissue, with the additional problem that the age/quality of the tissue is frequently inferior in human cases, compared to that of sheep thyroid tissue.

3. Unified Model of Proliferative Aspects of Thyroid Radiobiological Responses: Work continued on the development of a unified model of thyroid radiobiological response that brings together the following features: (i) Incidence of Hypothyroidism after Radioiodine Therapy; (ii) "Cell Survival" Properties as assessed in the Goitrgen Induced Weight Loss Assay; (iii) Cell Survival as assessed in Clonogenic Proliferative Assays in vivo; (iv) Limited data on Cell Kinetics available from the literature and inferred from experimental work.

While formal verification of a population structure of the H.F. type as defined by Wheldon et al. is not available, the available data is consistent with such a structure and it has been adopted as a working model.

3. (Continued):

The upper and lower bounds for each population compartment are inferred from a variety of experimental work in the literature, from our own laboratory, and from the work of Dumont and co-workers. Each compartment in the model may then be associated with one or more of the responses identified in (i) to (iii) above. Traditionally these responses have appeared to be at odds with each other and with the basic radiobiology common to other tissues. However, the model facilitates a coherent and self consistent explanation of all the properties without demanding any special radiobiological properties for the gland.

To achieve this coherence due account has had to be taken of the different population types present; the fundamental radiosensitivity of thyroid cells in clonogenic growth, and its dose rate dependance; the differing response of the gland to limited growth seen in the weight assay or in outgrowth from tissue explants; and finally the long term response of cells many years after irradiation as seen in radioiodine therapy. The figures and measurements associated with each step have been brought together as a unified whole, which hopefully will be confirmed during the coming year and strengthened by a more explicit insight into the underlying population structure.

4. Dosimetry and Risk Evaluation: A study has been initiated to determine the most appropriate physical parameter with which to correlate risk estimates in the thyroid. This involves the collection of data on gland mass, cell population structure, total energy deposited and risk per cell. The study has as its premise that it is unlikely that a fixed concentration of activity, e.g. 1μCi/g of tissue, which delivers a fixed absorbed dose, will produce the same risk in an 80g gland as it will in a 10g one.

IV. Objectives for the next reporting period:

1. To continue to investigate the apparent absence of transformation after 131-I and to identify possible reasons for this response in sheep thyroid tissue.
2. To continue similar investigations in human thyroid tissue, with some consideration given to the age of the donor, for each tissue sample.
3. To continue to develop a 3-D culture model for the thyroid, as a means of approaching the dosimetric problem of follicular cell irradiation.
4. To support the above studies with epidemiological and other studies where required.
5. To continue to develop a method for culture of small amounts of human tissue from needle biopsies of thyroids irradiated in vivo.
6. To evaluate an appropriate index as an alternative to dose which may be used as a denominator for risk in statements of risk estimates.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. C. Seymour et al., Radiobiology Laboratory, St. Luke's Hospital, Highfield Road, Rathgar, Dublin 6.

Prof. M. Cullen, St. James's Hospital.

Prof. J.E. Dumont, Hospital Erasme, Brussels.

Dr. P. Smyth, Woodview, University College, Dublin.

VI. Publications:

Radiation Response of the Thyroid 1: A New Model Integrating Survival Properties and Hypothroidism at the Cellular Level, 1988. In "Recent Advances in Radiation Biology". Ed: E. Riklis. (Weinheim, VCH Publishers) (in press)
J.F. Malone, M. Lewis and B. Tuohy.

Thyroid Radiation following Chernobyl Accident in CEC Countries: An Assessment of the Projected Fatal and Non-Fatal Harm, 1988. In "Recent Advances in Radiation Biology". Ed: E. Riklis. (Weinheim, VCH Publishers). (in press).
J.F. Malone and P. Gilligan.

Dosimetry and Consideration of Special Groups: In Utero, the Neonate, Children and Adults. (1988). In "Basis for Iodine Prophylaxis after Nuclear Accidents". (W.H.O., Copenhagen). (in press).

Development of a Unified Model to describe the response of the thyroid to radiation, (1988). Book of Abstracts - Joint Meeting on Experimental and Clinical Radiobiology - Netherlands Radiobiological Society.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor: Contract no.: BI6-D-096-F

Commissariat à l'Energie
Atomique
B.P. n° 6
F - 92265 Fontenay-aux-Roses

Head(s) of research team(s) [name(s) and address(es)]:

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Département de Protection
CEA-IPSN de Fontenay-aux-Roses
B.P. n° 6
F - 92265 Fontenay-aux-Roses

Telephone number: 01-654.73.26

Title of the research contract:

Experimental approach of absolute and relative risk concepts in radioinduced cancers. Role of combined effects.

List of projects:

1. Experimental approach of absolute and relative risk concepts in radioinduced cancers. Role of combined effects.

Title of the project no.: B16-D-096F

Experimental approach of absolute and relative risk concepts in radioinduced cancers. Role of combined effects.

Head(s) of project:

Dr R.Masse

Scientific staff:

M.Morin, J.Boncorps

I. Objectives of the project:

Application of the absolute risk concept implies that the increase in cancer in a certain population is constant and independant of age; regardless which age range is considered, the effect should be the same.
Relative risk imply that the spontaneous cancer incidence is multiplied with a certain factor. Since spontaneous cancer incidence depends on age, the excess induced cancer is also dependent on age.
Acceleration and initiation of tumours are interrelated by a quantitative relation which can be experimentally determined in rats for incidental tumours.
This study intends to verify these assumptions in control rats and in neutron or gamma irradiated rats. The combined effects of carcinogenic agents and promoters will also be characterized by a quantitative relation in agreement with the different hypotheses corresponding to the initiating and promoting mechanisms.

II. Objectives for the reporting period:

III. Progress achieved:

MATERIEL ET METHODE

1 - L'animal et ses cancers.
Les expériences ont été réalisées avec des rats Sprague-Dawley SPF âgés de 3 mois. Les animaux sont gardés jusqu'à leur mort naturelle, sauf pour les études de cofacteurs où un sacrifice systématique est pratiqué.
Après autopsie les prélèvements sont fixés dans du Bouin-Hollande, puis ils sont inclus en paraffine, coupés à 5 microns et colorés à l'hémalun-éosine-safran. La classification des tumeurs est effectuée selon une adaptation au rat de celle utilisée chez l'homme (ICD n°8).

2 - Conditions d'irradiation.
Les irradiations gamma sont faites avec des sources de Cobalt 60. Deux débits de dose sont utilisés: 0.1 Gy et 10 Gy par heure délivrés soit en chronique, soit en irradiation fractionnée. A 0.1 Gy par heure les doses varient de 1 Gy à 39 Gy avec un total de 951 animaux; à 10 Gy par heure la gamme de doses varie de 9.5 Gy à 18.5 Gy avec un total de 60 rats.
Les irradiations neutroniques sont pratiquées avec deux réacteurs différents. Pour les débits de dose élevés nous avons utilisé le réacteur Silène de Valduc, 380 rats ont été irradiés à des doses allant de 0.4 Gy à 2.86 Gy avec des temps de doublement de l'ordre de quelques millisecondes. Le réacteur Néréide de Fontenay-aux-Roses a permis d'irradier 1124 rats; les doses sont comprises entre 0.016 Gy et 8 Gy, le débit de dose variant de 0.1 cGy par heure pour les plus faibles doses à 10 cGy par heure pour les doses les plus élevées; l'irradiation était étalée sur des périodes allant de 1 jour à 6 semaines.
714 rats témoins sont suivis comme référence.
Les inhalations de radon 222 sont répétées cinq jours par semaine pendant plusieurs semaines ou mois, la dose totale est comprise entre 50 WLM et 2240 WLM.

RESULTATS

1 - Etude des animaux témoins.
Sur 714 animaux dont la moyenne de survie est de 837 jours, on a observé une incidence de cancers de 31%. 504 rats n'ont pas présenté de cancers, 201 rats ont 1 cancer et 9 rats ont 2 cancers.
On a observé 23 métastases, soit un taux de 10.5%. En plus des cancers on a trouvé 22% de tumeurs bénignes.

2 - Etude des effets de l'irradiation.
Ces effets dont l'intensité dépend de la dose et des modalités de l'irradiation sont au nombre de trois: un raccourcissement de la durée de la vie, une diminution du temps de latence des tumeurs, et une augmentation de la fréquence des cancers.
Avec les irradiations globales neutroniques ou gamma on constate que le raccourcissement de la durée de vie et la diminution du temps de latence des tumeurs malignes et bénignes ont pratiquement la même valeur. (tableau 1).
Le tableau 2 montre que pour les tumeurs malignes, la fréquence croît avec la dose alors que pour les tumeurs bénignes cette fréquence reste pratiquement constante sauf avec les forts débits de dose.
Sur la figure 1 on voit que pour la somme des cancers du rat (thyroïde exceptée) le modèle à prendre en compte pour l'estimation globale de l'effet est celui du risque relatif où l'excès de cancers dans chaque tranche d'âge est proportionnel au taux des cancers spontanés.

Après irradiation globale chez le rat, on observe qu'en fonction de la dose, les cancers des différents organes n'apparaissent pas de la même façon. Il existe, comme chez l'homme, des organes plus radiosensibles que d'autres à l'induction cancéreuse par l'irradiation. Cette radiosensibilité peut être analysée en fonction de deux critères de l'action des rayonnements: le raccourcissement du temps de latence et l'augmentation de la fréquence des cancers.
Par comparaison avec ce qui est observé chez les rats témoins, si on classe les organes radiosensibles en fonction du temps de latence, on voit que les organes les plus sensibles sont dans l'ordre: l'os, le poumon et le système hématopoïétique. Aucune différence fondamentale n'apparaît entre les carcinomes et les sarcomes considérés dans leur ensemble.
Si on classe les organes en fonction de la fréquence de leurs cancers, on observe un changement important avec le précédent classement: le système hématopoïétique se classe alors parmi les cancers à faible radiosensibilité, alors que le poumon et l'os gardent leur forte radiosensibilité. Ceci traduit une différence fondamentale entre le comportement des tumeurs du système hématopoïétique et celui des tumeurs solides.

3 - Etudes de synergie: initiation-promotion.

- La 5-6 naphtobenzoflavone (BNF).
L'action promotrice spécifique sur les carcinomes pulmonaires de la BNF, administrée par voie générale, a été mise en évidence après induction par des produits chimiques ou par irradiation. Ce phénomène ne touche que les cellules pulmonaires intervenant dans la genèse des carcinomes épidermoïdes, il ne se produit que si l'administration de BNF suit l'irradiation et il entraîne l'apparition rapide (en moins de 6 mois) de tumeurs épidermoïdes. Ce phénomène ne s'observe que lorsqu'une combinaison critique entre la dose d'irradiation, ou de produit, et la dose de BNF a été atteinte. (tableau 3).
Dans ces expériences l'irradiation se comporte comme un initiateur, et pour être efficace elle doit être délivrée avant la BNF. Pour une dose constante de benzoflavone la combinaison critique dose d'initiation-dose de promotion est atteinte d'autant plus rapidement que la dose d'irradiation est plus élevée.

- Fibres et poussières.
L'injection intratrachéale de diverses particules en suspension dans du sérum physiologique est effectuée sur 170 rats un mois après la fin de l'inhalation pendant deux mois de radon 222 à la dose de 1600 WLM.
Aucune différence importante d'incidence tumorale n'a été trouvée dans les différents groupes par rapport au groupe de rats exposés seulement au radon. (tableau 4).
Les fibres et poussières ne sont pas promotrices pour les cancers pulmonaires alors qu'elles se révélaient initiatrices avec la benzoflavone.

4 - Analyse statistique des données.
Cette analyse a été réalisée par M.Dalebroux. Dans un premier temps, l'ensemble des cancers a été étudié, puis les carcinomes et les sarcomes ont été séparés, et pour finir on a comparé les carcinomes pulmonaires à l'ensemble des carcinomes.
Chez le rat, comme chez l'homme, l'excès de cancers par tranches d'âge augmente avec la survie.
Si on étudie les relations entre le logarithme de la fréquence et l'âge, pour les différentes doses, les cancers se répartissent suivant une droite dont la pente croît avec la dose. Le risque ne peut donc pas être de type absolu. Le mode de risque relatif est plus proche de la réalité mais ne donne pas une représentation parfaitement correcte du phénomène.

A faibles doses l'apparition des tumeurs dans le temps est sensiblement la même pour les neutrons et les gamma, ce qui laisse supposer l'existence d'un même phénomène pour des rayonnements différents.
Pour une dose d'irradiation déterminée, la vitesse d'évolution des carcinomes en fonction de l'âge est toujours supérieure à celle des sarcomes.
Lorsqu'on sépare les sarcomes et les carcinomes en deux sous-groupes comportant pour l'un les organes profonds et pour l'autre les tissus mous, on obtient dans les deux cas des pentes comparables.
Il n'est pas possible d'analyser statistiquement chaque organe séparément car le nombre de données est insuffisant. Cette analyse n'a pu être faite que pour les cancers pulmonaires, et ceci a permis de comparer les irradiations par le radon aux irradiations produites par les neutrons et les gamma. Les carcinomes pulmonaires ont un comportement comparable à celui de l'ensemble des autres carcinomes.

DISCUSSION

La protection radiologique est basée sur l'utilisation de relations dose-effet. Pour l'induction cancéreuse, une relation linéaire sans seuil est considérée fournir une description correcte des données. En temps que modèle elle est compatible avec la théorie selon laquelle le cancer est la conséquence directe de la transformation radio-induite d'une seule cellule, comme le suggère les transformations cellulaires "in vitro".
Les données acquises chez le rat montrent que les relations dose-effet présentent en réalité, pour les gammes de doses utilisées, une concavité tournée vers le haut; les doses totales les plus faibles représentent des doses voisines des doses maximales admissibles annuelles pour les travailleurs en ce qui concerne le radon et les neutrons, ce qui exclut un effet de stérilisation par impacts multiples.
Avec les gamma, à partir de la dose minimale délivrée (1 gray) avec un débit relativement élevé (0.1 gray par heure), la relation est également à concavité tournée vers le haut mais un effet létal est probable. Une expérience en cours faite à 1 Gy et avec un débit de 1.5 mGy par heure ne semble pas, pour le moment, apporter de résultats qualitativement différents.
La forme des courbes à concavité tournée vers le haut n'est pas compatible avec la notion que l'atteinte d'une seule cellule, évoluant indépendamment du tissu, est responsable de la tumeur maligne. Un rôle doit être attribué au niveau tissulaire où cohabitent plusieurs populations cellulaires d'origine et de fonctions différentes.
Quels que soient les mécanismes sous-jacents, le fait que, pour les trois types de rayonnement, les courbes soient de formes comparables, facilite le calcul des facteurs d'efficacité biologique relative (EBR). L'EBR pour les neutrons par rapport aux gamma du cobalt 60 est, aux plus faibles doses et pour tous les cancers, de l'ordre de 50. Pour le radon, 1 EBR : WLM/cGy gamma, serait aux plus faibles doses pour les cancers pulmonaires de l'ordre de 5.
Les expériences de synergie avec la 5,6 naphtoflavone montrent que l'apparition des tumeurs est une fonction du produit des doses des deux agents (mg/kg et WLM). Le fait que l effet synergique radiation-benzoflavone au niveau pulmonaire soit multiplicatif permet de comparer rapidement le potentiel des cancérogènes initiateurs chimiques déposés dans le poumon avec une dose d'irradiation. Connaissant la déposition et la clairance pulmonaire on peut calculer les limites de concentration susceptibles d entraîner un même risque à partir d une définition comparable de la dose pour les toxiques cumulatifs.

Tableau 1 - Evolutions de la durée de vie et du temps de latence des tumeurs en fonction de la dose.

	DOSE (Gy)	DUREE DE VIE (jours)	TEMPS DE LATENCE DES TUMEURS (en jours après la naissance) MALIGNES (sans la thyroïde)	BENIGNES
TEMOIN	0	803	805	845
	0	852	819	877
NEUTRON	0.016	838	848	882
(Triton)	0.08	755	770	768
	0.4	642	648	697
	1.5	565	576	626
	2.4	536	552	581
	3.5	491	518	540
	4.4	471	490	483
	5.6	446	454	480
	8.0	329	328	-
(Silène)	0.4	638	635	678
	1.15	575	630	661
	1.73	536	563	609
	2.0	506	534	510
	2.86	541	579	573
GAMMA	1.0	790	814	822
(Co 60)	1.0	786	795	839
0.1 Gy/h	3.0	738	774	784
	6.0	657	668	723
	10.0	621	589	659
	12.0	591	596	710
	13.0	581	630	615
	16.5	548	581	604
	18.0	480	549	557
	20.0	425	474	499
	26.0	288	430	427
	28.0	480	551	589
	31.0	126	296	-
	39.0	137	-	148
(Co 60)	9.5	483	507	559
10 Gy/h	12.0	557	625	579
	19.0	234	495	-
	24.0	118	-	-
	28.5	127	-	-

Tableau 2 - Evolution du nombre des tumeurs en fonction de la dose.

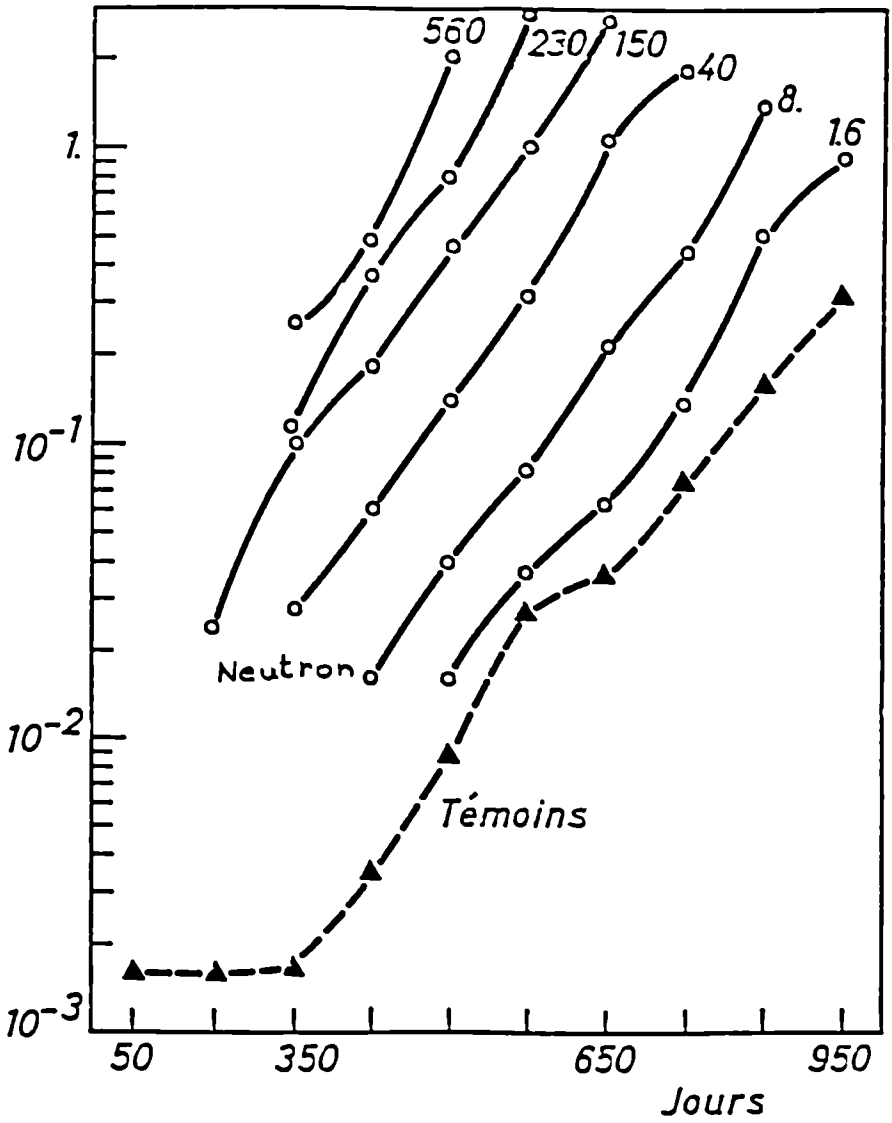
	NOMBRE DE RATS	DOSE (Gy)	TUMEURS TOTALES malignes	TUMEURS TOTALES bénignes	TUMEURS SANS THYROIDE malignes	TUMEURS SANS THYROIDE bénignes
TEMOINS	218	0	61	54	45	35
	496	0	158	103	86	85
NEUTRON (Triton)	300	0.016	158	57	119	54
	158	0.08	109	22	92	22
	150	0.4	140	30	132	30
	101	1.5	102	30	97	29
	120	2.4	142	31	138	31
	80	3.5	87	17	84	17
	40	4.4	43	6	39	6
	40	5.6	37	9	37	9
	20	8.0	11	0	11	0
(Silène)	116	0.4	102	37	94	37
	80	1.15	116	73	94	54
	40	1.73	54	32	48	25
	104	2.0	176	23	171	23
	40	2.86	46	18	44	14
GAMMA (Co 60) 0.1 Gy/h	204	1.0	158	86	100	63
	301	1.0	205	214	137	114
	120	3.0	89	39	69	32
	50	6.0	38	23	34	22
	30	10.0	27	6	24	6
	40	12.0	38	9	37	7
	20	13.0	12	6	12	6
	36	16.5	37	10	37	10
	40	18.0	27	11	25	11
	20	20.0	7	6	7	6
	20	26.0	11	1	11	1
	30	28.0	25	3	25	3
	20	31.0	1	0	1	0
	20	39.0	0	1	0	1
(Co 60) 10 Gy/h	20	9.5	14	9	12	9
	20	12.0	18	5	17	5
	20	19.0	7	0	6	0
	20	24.0	0	0	0	0
	20	28.5	0	0	0	0

Tableau 3 - INCIDENCE DES CANCERS PULMONAIRES CHEZ LE RAT SPRAGUE-DAWLEY APRES ACTION COMBINEE D'UNE INITIATION PAR IRRADIATION OU SUBSTANCE POTENTIELLEMENT CANCEROGENE ET D'UNE PROMOTION SPECIFIQUE PAR LA BENZOFLAVONE (BNF).

		NOMBRE D'INJECTIONS INTRA-MUSCULAIRES DE BNF (25 mg/kg par injection)										
		0	1	2	3	4	6	7	8	10	12	16
RAYONNEMENT	DOSE											
0	0	0					0	0	0	0	4	20
NEUTRONS	15	0										0
(cGy)	23	0										10
	30	0									30	
	45	0									40	
	75					0	0	0	50			
	150					0	25	50	75			
	220				25	0	25				90	
GAMMA	400										0	
(COBALT 60)	800						0				0	
(cG/)	1200					0				25		
	1600				0		40					
RADON	50	0	0							12		
(WLM)	100								12			
	225		0					25	25		50	
	450					10			37			
	500	0				20						
	1000		12				50				80	
	1200		25	30		45						
	1500	12	12			60						
	2000	15	20	50		75					100	
	2240		80			100	100				100	
FIBRES (mg)												
Crocidolite	5								38			
	10								65			
Chrysotile	10								17			
Quartz DQ12	5								25			
	10								90			
BERYLLIUM (mg)	0.6								33			
CHROMATES (mg)												
Plomb 1	12								50			
Plomb 2	20								100			
Plomb 3	20								70			
Strontium	10								17			
Zinc	10								17			
BENZOPYRENE (mg)												
	3								17			
	5								80			

Tableau 4 - Action combinée au niveau du poumon d'une irradiation à 1600 WLM de radon et d'une injection intratrachéale de fibres ou poussières minérales.

	nombre de rats	rats avec cancers pulmonaires	cancers pulmonaires	survie (jours)
radon seul	36	22 (61%)	23 (64%)	748
radon + sérum physiologique	17	14 (82%)	18 (106%)	748
radon + fibres verre 20 mg	15	12 (80%)	19 (127%)	671
radon + amosite 10 mg	17	12 (71%)	14 (82%)	696
radon + chrysotile 10 mg	18	12 (67%)	13 (72%)	759
radon + béryllium 0.6 mg	17	11 (65%)	16 (94%)	715
radon + crocidolite 10 mg	16	10 (62.5%)	11 (69%)	660
radon + hématite 20 mg	18	11 (61%)	16 (89%)	751
radon + quartz 20 mg	18	11 (61%)	14 (78%)	705



_Tous les cancers: incidence par tranche d'âge.

Figure 1

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

V. Publications:

- Rapports DPS/IPSN: 1986 pp.185-186, 191-192
1987 Etude de l'action des faibles débits de dose.

- Michèle Morin, Jacques Lafuma. Rapport CEA-R-5462: septembre 1988. Les cancers radio-induits du rat. Etude expérimentale.

- Masse R., Morin M., Luccioni C., Lafuma J.
Quality factors for neutrons biological data. Sixth Symposium on Neutron Dosimetry, Neuherberg, 12-16 octobre 1987, Abs.(87-28).

- Morin M., Chameaud J., Masse R., Lafuma J.
Cocarcinogenic effects of high-LET radiation at low doses, comparison with gamma-rays effects. Congress Radiation Research, Edinburgh, 1987, D 43-11 V, Abs.(87-43).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-235-UK

United Kingdom Atomic Energy
Authority UKAEA
11 Charles II Street
GB - London SW1Y 4QP

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Morgan
Environ. & Medical Sciences Div.
Harwell Laboratory
Didcot
GB - Oxon OX11 ORA

Telephone number: 0235-24141

Title of the research contract:

Consequences to lung and bone of exposure to actinides.

List of projects:

1. Synergistic effects of cigarette smoke in the induction of lung tumors by inhaled $^{239}PuO_2$.
2. Studies on the distribution and effects of alpha and beta emitting radionuclides on the bone and the bone marrow.

Title of the project no.:

Synergistic effects of cigarette smoke in the induction of lung tumours by inhaled $^{239}PuO_2$.

Head(s) of project:

A Morgan

Scientific staff:

A Morgan
N D Priest
R J Talbot
A Black

I. Objectives of the project:

To demonstrate that exposure to cigarette smoke enhances the incidence of lung tumours in mice previously exposed to $^{239}PuO_2$.

II. Objectives for the reporting period:

To complete a study started under a previous contract (B16-D-100-UK) comparing the incidence of lung tumours in plutonium-exposed CBA/H mice which had received one years exposure to cigarette smoke with that in animals which were either sham-exposed to smoke or were given no treatment other than their initial exposure to plutonium. To initiate further studies designed to determine the effects of tobacco smoke inhalation by mice on plutonium induced lung tumours.

III. Progress achieved:

1. METHODOLOGY

Female CBA/H mice were exposed (nose only) to an aerosol containing $^{239}PuO_2$ with an AMAD of 1.5 μm and g of 1.3. Each animal was approximately 10 weeks of age at inhalation and received an initial alveolar deposit (IAD) of about 100 Bq of ^{239}Bq. At 8 days after inhalation the animals were randomly allocated to one of three treatment groups. One group, designated PTS, was exposed to tobacco smoke for a period of one year. Each animal received tobacco smoke - from a commercial, U.K., middle-tar brand of tipped cigarette at a concentration of approximately 1.3 mg l^{-1} of tar particulate material for one hour per day, five days each week, for one year. A second group, designated PSS, were sham-exposed according to the protocol described for PTS. The third group received no further treatment and were allocated to the cage control group - PCC. Following the cessation of smoking the animals were returned to the animal maintenance facility for a further six month period during which time the animals received no further treatment. At the end of this period the lungs of the mice were examined at low magnification by transmitted light microscopy and any lung lobes containing opacities (mostly tumours) were excised and embedded for histopathological examination.

In addition, a second series of experiments have been initiated using the methods described above, but with some modifications. These include the use of 2 lower IAD's of plutonium (~25 and ~50 Bq) and cigarettes yielding more tar. In addition, extra control groups have been included in the study which is being conducted to GLP standards. This study is being funded jointly by British Nuclear Fuels plc and the CEC.

2. RESULTS

Examination of the lungs of the animals assigned to the first study revealed 65 macroscopically defined lesions. Most of these lesions were very small and many would probably have been missed using conventional histopathological techniques. For example, 57% of the lesions were less than 1.5 mm in diameter and 22% were less than 1 mm. The lesions were distributed throughout the lung parenchyma and no association with the major airways was found.

The numbers of lesions found in each experimental group are shown in Table 1. It can be seen that of the 61 lesions of nodular appearance, 30 were found in the cage-control animals (PCC), 21 in the sham-smoked animals (PSS), but only 10 in the mice which received tobacco smoke (PTS). A similar trend was found when the percentage of the affected animals in each group were calculated: PCC - 54%, PSS - 51%, PTS - 26%.

Experimental Group	Number of Mice	Number of Nodules	% with Nodules	Alveolar Bq at Sacrifice
PCC	43	30	53.7	3.3 Bq
PSS	45	21	51.4	3.8
PTS	38	10	26.3	14.2

Table 1. Summary of the results of the macroscopic investigation of the numbers of lesions in the cage-control (PCC), sham-exposed (PSS) and tobacco smoke-exposed (PTS) mice.

However, the histopathological examination of the animals yielded a slightly different picture (see Table 2) as some of the nodules identified were not tumours. Nevertheless, the tobacco-smoked animals continued to show a deficit of tumours compared with the plutonium-exposed, cage-control animals. Interestingly, no malignant tumours were found in the tobacco-smoke exposed animals.

	Cage Control	Sham-Smoked	Tobacco-Smoked
Solid Alveolar Benign	6	4	3
Mixed/uncertain "	1	3	0
Mixed/uncertain Malignant	1	0	0
Tubular Bronchiolar Benign	7	8	7
Tubular Bronchiolar Malignant	6	1	0
Metastases			
Extra-pulmonary origin	4	1	0
% MICE WITH TUMOURS	46.5%	33.3%	26.3%
TUMOURS PER MOUSE	0.58	0.36	0.26

Table 2. Summary of the results of the histopathological examination of the nodules identified in Table 1.

As yet no results are available for the second series of experiments, although animals are being sacrificed periodically to determine the plutonium content of their lungs. The first animals will be sacrificed and examined for lung tumours in 1990.

IV. Objectives for the next reporting period:

During the forthcoming year it is intended to continue with the second synergy study using the lower dose of plutonium - to minimise cell sterilisation - and using cigarettes with a higher tar yield. It is also intended to complete a revised dose response study for $^{239}PuO_2$ using a range of IADs from 50-350 Bq over the longer 18 month period.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None.

VI. Publications:

Title of the project no.:

Studies on the distribution and effects of alpha- and beta-emitting radionuclides on the bone and bone marrow

Head(s) of project:

A Morgan

Scientific staff:

N D Priest
A Morgan
G R Morgan
J P Kellington

I. Objectives of the project:

1. To determine the comparative distribution of plutonium and americium in the bones and bone marrow of baboons, following their administration in different chemical forms by injection and inhalation.

2. To study the effects of bone turnover and growth on the redistribiution of plutonium and americium, and the influence of macrophages on their distribution in the red bone marrow.

3. To use the data obtained to construct realistic models for the dosimetry of bone-seeking radionuclides in the human skeleton.

II. Objectives for the reporting period:

- To continue to examine, by autoradiography, the bones of plutonium and americium contaminated baboons..

- To undertake a quantitative analysis of the distribution of plutonium in baboon bone at different times after its injection.

- To apply the dosimetric model developed for plutonium in man and to estimate the risks of leukaemia following plutonium intakes.

III. Progress achieved:

The project concerns studies currently being financed by the CEC, the UK Department of Health and the Underlying Programme of the UKAEA. They are being undertaken in association with the Instutite de Protection et Surete Nucleaire, CEA, France. The aim of the studies is to provide information on the way in which actinides deposit and redistribute in the skeleton of a primate closely related to man. This information has been used, and will continue to be used, to model the likely behaviour of plutonium and americium in man. It is hoped, in this way, to provide data enabling valid predictions of the dosimetry and toxicity of these elements to be made. The studies are an extension of previous work undertaken by the National Radiological Protection Board. The particular aspects of the work which have been studied most closely are those which relate to the risk of osteosarcoma and of leukaemia in plutonium-contaminated people.

1. Autoradiographic Studies

A quantative assessment of autoradiographs produced from ^{239}Pu-contaminated baboon femora and vertebrae has been undertaken. Bone removed from animals killed at either 1 day, 1 month, 3 months or 1 year after the injection of a plutonium-TBP complex has been examined. Autoradiographs were assessed in order to determine a) the fractional volume of different bone components in each bone examined, b) the bone turnovers rates and patterns in bone at different sites and c) the radionuclide distribution pattern. The results showed that plutonium was mostly deposited on

Bone Sample	One Day		One Year	
	PROXIMAL FEMUR (%)	VERTEBRAE (LUMBAR) (%)	PROXIMAL FEMUR (%)	VERTEBRAE (LUMBAR) (%)
Trabecular Surface	75	40	20	37
Volume	6	5	65	21
Cortical Surface	3	14	2	7
Volume	0.6	2	9	30
Bone Marrow	16	39	5	5

Table 1. The distribution of plutonium in baboon bones at one day and one year after the admninistration of a Tri-butyl phosphate complex

trabecular bone surfaces, but with about 10% in the bone volume and 16 to 40% in the bone marrow. Only 5-10% of the plutonium was concentrated by cortical surfaces. This result suggests that in most respects the initial deposition pattern of plutonium in the baboon is similar to that described for rodents. However, the plutonium-TBP complex, (but not plutonium citrate) was concentrated by macrophages in the bone marrow. At later

times after plutonium administration the bone growth and turnover processes redistributed the plutonium. This effect was most marked in the metaphyseal regions of the femur and least marked in the vertebrae. At one year after intake most plutonium present in the baboon femur was located in the volume of the trabecular bone. However, in the vertebrae much plutonium had been transferred to cortical bone (see Table 1). Animals killed after 1 and 3 months showed intermediate radionuclide distributions. Plutonium concentrations in the bone marrow descreased rapidly after administration then levelled out at about 5% of the skeletal content.

When the radiation doses received by the bone volume, by the bone surfaces, by the edges of the bone marrow and by deeper regions of the bone marrow were calculated, using the plutonium distribution data described above, it was found that ICRP dosimetric assumptions would have overestimated the bone surface doses, and bone marrow doses by a factor of about 2. Moreover, calculations made assuming plutonium to be volume distributed throughout the bone volume would have been equally inaccurate. An example of the dose distributions calculated is shown in Table 2.

	Tracks	ICRP	Vol
Bone Volume	72	50	46
Bone Surfaces	12	25	3
Marrow Edge	12	50	2
Deep Marrow	5		50

Table 2. A Comparison of the percent dose distributions calculated, for the baboon vertebra at one year after plutonium administration, either from the measured plutonium distribution, from measurements of fractional bone volume assuming volume radionuclide distribution or assuming the fractions recommended for use by the ICRP.

2. Dosimetric Modelling

The bone dosimetric model developed to describe the dosimetry of plutonium in the human skeleton has been applied and used to calculate the expected frequency of leukaemias in human populations of different ages. The risks of developing leukaemia have been calculated for individuals aged either 0, 10 or 20 years at intake. Risk estimates used for the calculations were derived from the results of the epidemiological studies of human populations exposed to either radium-226 or to Thorotrast. The results of the calculations are shown in Table 3.

Age at Intake	lower estimated of risk	upper estimate of risk	ICRP
0	.046	1.15	1
10	.0098	.196	1
20	.0076	.152	1

Table 3. The life-time risk of developing leukaemia following intakes of plutonium, relative to those calculated using ICRP methods and assumptions.

It can be seen that the present calculations suggest that ICRP methods tend to overestimate risk for intakes at all ages, but that these overestimates seem particularly great for adults.

IV. Objectives for the next reporting period:

- To continue the quantitative analysis of autoradiographs of baboon bone contaminated with plutonium.
- To publish the results of a pig study using radium.
- To continue to develop dosimetric models for bone-seeking radionuclides and to integrate these models with the new ICRP lung model.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr H Métivier
Section de Toxicologie et Cancerologie Experimentale
CEA-IPSN
BP 12. F-91680, Bruyeres-le-Chatel
France.

Dr R A Schlenker
Argonne National Laboratory
4700 South Cass Ave
Argonne, Illinois,
USA

VI. Publications:

Priest N.D. (1987) The metabolism and dosimetry of plutonium. In: The proceedings of CEIR meeting, 21st November 1986. Int. J. Radiol. Protect.

Priest N.D. (1989) Sensitivity testing of an age-related, multi-comparment dosimetric model for bone surface-seeking radionuclides in man. Accepted for publication by Health Physics.

Priest N.D. (1989) A personal evaluation of the risks of leukaemia following intakes of plutonium. Presented at the DOE/CEC Radium and Thorotrast Meeting, Bathesda Maryland U.S.A., 3-5 October, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-092-IRL

St. Luke's Hospital
Highfield Road
Rathgar
IRL - Dublin 6

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Mothersill
St. Luke's Hospital
Highfield Road
Rathgar
IRL - Dublin 6

Telephone number: 01-974552

Title of the research contract:

Inter-related studies on dose dependence and mechanisms of radiation induced carcinogenesis and environmentally induced and radiation promoted carcinogenesis.

List of projects:

1. Radiation transformation in primary culture systems.

Title of the project no.:

Radiation transformation in primary culture systems

Head(s) of project: Dr C. Mothersill
Dr C. Seymour

Scientific staff: Dr C. Mothersill
Dr C. Seymour
Ms A. Cusack
Ms M. McDonnell
Ms C. O'Donnell
Ms P. Nolan

I. Objectives of the project:

(1) To develop methods for culture of tissues of interest in the radiation protection field.

(2) To study the induction by radiation of features associated with carcinogenesis in these culture systems.

(3) To investigate mechanisms involved in the development of selected radiation transformation endpoints in the culture systems.

(4) To attempt to quantify radiation transformation frequencies in selected primary culture systems.

(5) To investigate the potentiating effect of known tumour promotors such as phorbol esters.

II. Objectives for the reporting period:

(1) To continue the autoradiographic and cytochemical analysis of irradiated cultured normal human epithelial cells.

(2) To study the response of cultured malignant tissue in order to establish a profile of positive control endpoints.

(3) To continue to develop methods for microanalysis of LDH, GST and malignant prekeratins.

(4) To continue to study the relevance of lethal mutations to studies of transformation frequency.

(5) To continue ultrastructural studies.

(6) To continue to develop 3-D models of available normal epithelial tissue.

III. Progress achieved:

Methodology

(1) Immunocytochemical techniques are now being applied in situ to cultures. This permits accurate localisation within the culture of various components. Techniques are being learned which allow this method to be used with electron microscopy (immunogold staining), allowing very accurate spatial localisation of components in cells. The in situ nature of the method means that cell types can be identified and studied separately.

(2) Antibodies against LDH isoenzymes 1 and 5 have been obtained and tested. Assays for GST are still limited by lack of suitable assay methods for low cell numbers but work with malignant prekeratins has developed to the level where gels can be scanned and changes in the different keratins identified and quantified. Specific transformation markers such as βHCG are also being examined and a source of antibodies to the cMyc and cFos oncogenes has been identified.

(3) New methods for optimising the growth of the cultures have been tried. These included a nutrient optimisation and matrix optimisation. Collagen gel techniques are still being tested and a new shrinking floating gel technique which promotes a more normal polarity and shape for epithelial cells appears promising. The gels are amenable to careful sectioning and permit examination of the 3-D structure of the cultures.

(4) In an effort to obtain more regular supplies of normal tissues for culture, available techniques were applied to biopsy sized pieces of normal tissue, obtained during investigative procedures in hospital.

Results

(1) Immunocytochemical techniques: These became the principal methods for screening cultures for radiation induced changes and proved very successful. While the method is not quantitative at present, it gives information on presence or absence of agents in a cell or cell type. An instrument capable of performing quantitative analyses of cellular concentrations of agents has been obtained (Becton & Dickenson Cell Analysis System) and this area is expected to undergo major expansion during 1989. To date an increase in proliferation of normal oesophageal, urothelial and endometrial epithelial cells exposed to low dose irradiation ($\leq$2.5 Gy), has been seen using Ki67, an antibody against proliferating cell nuclear antigen. This can be quantified and is statistically significant. A selective and very great proliferation of endothelial cells and their subsequent organisation into capillaries is also very apparent 1 - 2 weeks following exposure of the explant culture to doses $<$5 Gy. This effect is not seen at doses greater than 5 Gy, where cell death predominates. A third type of cell which has the ultrastructural appearance of a well differentiated squamous carcinoma cell (very small size, irregular nucleus, high density of tonofibrils and many nuclear pores) has been detected in cultures from normal (non SCC) oesophagi following low dose irradiation. This cell type occurs at a level of 1 - 2% in cultures and the cells generally proliferate rapidly to form foci on the epithelial monolayer. The cells are positive for both epithelial and endothelial immunochemical markers.

(2) Ultrastructure: A quantitative analysis of several parameters indicative of cellular damage, ageing or alteration was performed. Results indicate (i) premature ageing of cells in irradiated cultures which survive. The effect predominates at doses in the range 2.5 - 5 Gy; (ii) a very great

increase in nuclear irregularities both with dose (peaking at 5 Gy and then falling off) and time after irradiation, ranging from ~5% of cells in the controls stable over a 2 week period to over 45% for cells irradiated to 5 Gy and maintained for 2 weeks. An increase in nuclear inclusions and in % giant cells was also seen to peak at 5 Gy. Margination of the chromatin showed a significant inverse relationship with dose.

(3) Biochemistry: The continuing problem of low cell numbers has hampered development in this area but antibodies to LDH 1 and 5 have permitted this enzyme to be monitored *in situ* in the cultures. Results confirm the suspected shift to the anaerobic isoenzyme form and quantification of the change is under way. Normal endometrial cultures are being routinely checked following irradiation for expression of βHCG, a tumour marker, and although positive results have been obtained, the positive cells do not selectively proliferate. Cytokeratin analysis has been performed for control and irradiated normal oesophageal cultures and for positive control squamous cell carcinoma tissue and cultures. Results indicate that cytokeratin No. 18, found in the tumours, occurs only in irradiated normal cultures (see Contract B16 184 IRL).

(4) Lethal mutations: Following the suggestion that lethal mutations would lead to underestimations of transformation frequency in clonogenic survival dependent assays, it became important to determine (i) the level of lethal mutation expression in transformed *versus* normal cells and (ii) whether expression of lethal mutations occurred in all progeny from a damaged progenitor cell or only in some. The results of experiments suggest that cells composing transformed foci of C3H 10T½ cultures do not express lethal mutations. This observation means that underestimations of transformation frequency/surviving cell are even greater than previously postulated. Regarding the lineage committment of the lethal mutation, it appears from autoradiographic and immunocytochemical analysis of surviving clones that some but not all cells in most surviving clones contain a lethal mutation.

(5) 3-D culture: Progress in this area has been slow due to the demanding nature of the technique but cultures have been established using a modification of the collagen gel technique which permits shrinkage of the gel after cells have grown in it. This promotes the development of differentiation. Technical problems associated with processing the gels for histological examination have been overcome but the technique requires exceptional care and skill.

Discussion

The success of immunocytochemical analyses has made it possible to get cell type specific information with the very low cell numbers available. Extension of the technique to include ultrastructural preparations and planned quantitative analysis should make it possible to identify very subtle and localised changes in irradiated cultures. Development of 3-D models provides a means of building up a picture of radiation induced cell changes in mixed, differentiated cultures where cells are organised and in contact. The results of attempts to identify markers of transformation strongly suggest that enhanced proliferation, coupled with the appearance of cytokeratin band 18 and of ultrastructural changes in the nucleus of the cells are seen in tumour controls and in irradiated normal cultures but not in unirradiated normal cultures. The changes in the proliferation of endothelial cells following irradiation may also be significant.

IV. Objectives for the next reporting period:

(1) To integrate immunocytochemical and ultrastructural analysis of irradiated tissues in an attempt to quantify cellular changes.

(2) To develop a mathematical model for radiation induced transformation expression which incorporates a lethal mutation term.

(3) To continue to develop 3-D culture systems for available tissue.

(4) To quantify the biochemical changes detected in isoenzymes and intermediate filaments on a per Gray per cell basis following irradiation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr R. Hodgekiss, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, U.K.

Dr T. Connors, M.R.C. Toxicology Unit, Carshalton, Surrey, U.K.

Dr Y. Ramsey, Zoology Department, University College, Belfield, Dublin 4.

Prof. N. Fusenig, German Cancer Research Centre, Heidelberg.

VI. Publications:

Mothersill, C., Cusack, A. & Seymour, C.B. (1988). Radiation-induced outgrowth inhibition in explant cultures from surgical specimens of five human organs. Brit. J. Radiol., 61, 226-30.

Seymour, C.B., Mothersill, C., Cusack, A. & Hennessy, T.P. (1988). The effect of radiation on the growth of normal and malignant human oesophageal explant oultures pre-treated with bleomycin. Brit. J. Radiol., 61, 383-7.

Seymour, C.B. & Mothersill, C. (1988). The effect of glycolysis inhibitors on the radiation response of CHO-K1 cells. Radiat & Environ. Biophys. 27, 49-57.

Seymour, C.B. & Mothersill, C. (1988). Re. Clonogenicity of the progeny of surviving cells after irradiation, by R.Born and K.R.Trott (1988). Letter to the Editor, Int. J. Radiat. Biol., 54 (3), 497-502.

Mothersill, C. & Seymour, C.B. (1988). Letter to the Editor, Int. J. Radiat. Biol. (in press).

Alper, T., Mothersill, C. & Seymour, C.B. (1988). Lethal mutations attributable to misrepair of Q lesions. Int. J. Radiat. Biol., 54, 525-30.

Seymour, C.B. & Mothersill, Carmel (1988). Radiation transformation studies: are they relevant to radiation protection problems? J. Radiol. Prot., 8 (3), 129-38.

Mothersill, C., Cusack, A., MacDonnell, M., Hennessy, T.P. & Seymour, C.B. (1988). Differential response of normal and tumour oesophageal explant cultures to radiation. Acta Oncol., 27 (3), 275-80.

Alper, T., Mothersill, C. & Seymour, C.B. (1988). Reply to letter by M.M. Elkind et al., Int. J. Radiat. Biol., 53 (5), 861-3.

Abstracts:

McDonnell, M., Mothersill, C., Seymour, C.B., Cusack, A., Moriarty, M. & Hennessy, T.P. (1988). Effect of Novanthrone on the survival of oesophageal explants in culture. Proc. Irish Association for Cancer Research, Galway.

Mothersill, C., Seymour, C.B. & Alper, T. (1988). The relationship between lethal mutations and the occurrence and size of the radiation survival curve shoulder. British Institute of Radiology Work in Progress Meeting, London.

Mothersill, C., Seymour, C.B., Cusack, A., McDonnell, M. & Moriarty, M. (1988). Effect of some carcinogens in combination with radiation on normal human primary bladder and oesophageal cultures. Int. J. Radiat. Biol., 53 (6), 1004.

Seymour, C.B. & Mothersill, C. (1988). The radiation survival curve shoulder split dose recovery and error prone repair - a connection? L. H. Gray Conference, Oxford.

Mothersill, C., Seymour, C., Cusack, A. Hennessy, T.P. & Moriarty, M. (1988). An attempt to develop a useful model for optimising cancer therapy. Cell Tissue and Organ Culture Society Meeting, Gent.

Mothersill, C., Seymour, C.B., Cusack, A., Hennessy, T.P. & Moriarty, M. (1988). Development of a technique for optimising cancer therapy using a human epithelial model system. Third Scientific Meeting of the British Oncological Association, York.

Mothersill, Carmel & Seymour, Colin (1988). Response of a normal human epithelial culture model to carcinogen exposure. 36th Meeting of European Tissue Culture Society, Gent.

Seymour, C., Mothersill, C., Cusack, A., Hennessy, T.P. & Moriarty, M. (1988). Development of a technique for optimising cancer therapy using a human epithelial model system. 7th Annual Meeting of European Society for Therapeutic Radiology and Oncology, The Hague.

Papers in press or submitted:

Mothersill, C., Seymour, C.B. & Moriarty, M. Development of radiation transformation systems for epithelial cells - problems and perspectives. Proc. 14th L.H.Gray Conference on the Biological Effects of Low Doses of Ionising Radiation (Taylor & Francis Ltd., in press).

Mothersill, C., Seymour, C.B., Cusack, A., O'Brien, A. & Butler, M. The effect of radiation and platinum analogues on the growth of normal and tumour bladder explant cultures. Acta Oncologica (submitted).

Mothersill, C., Cusack, A. & Seymour, C.B. Enhanced proliferation of cells from human tissue explants following irradiation in the presence of environmental carcinogens. Radiat. & Environ. Biophys. (submitted).

Seymour, C.B. & Mothersill, C. Lethal mutations, the survival curve shoulder and split dose recovery. Int. J. Radiat. Biol. (under revision).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Institut de Protection et de
Sûreté Nucléaire
CEN de Fontenay-aux-Roses
B.P. n° 6
F - 92265 Fontenay-aux-Roses

Contract no.: BI6-D-101-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. N. Parmentier
CEN-IPSN de Fontenay-aux-Roses
B.P. n° 6
F - 92265 Fontenay-aux-Roses

Telephone number: 1-4654 70 80

Title of the research contract:

Lung modelling contribution: deposition and clearance studies in man.

List of projects:

1. Experimental studies and modelling of deposition and retention of inhaled aerosols in man.

Title of the project no.:

EXPERIMENTAL STUDIES AND DEPOSITION MODELLING OF INHALED AEROSOLS IN MAN

Head(s) of project:

Docteur N. PARMENTIER

Scientific staff:

M. ROY, C.E.A. engineer, with the collaboration of the medical staff of the respiratory function exploration Department Pitié-Salpétrière Hospital PARIS.

I. Objectives of the project:

Calculation models for assessment of doses to the lung and other organs, and the derivation of limits of intake of inhaled toxicants and radioactive substances, include the aerosol deposition and retention variables in the human respiratory tract.

It is well known that deposition of inhaled aerosols is determined by the physical-chemical characteristics of the particles, the airway geometry, and the breathing pattern. This work deals with experimental studies of deposition of inert particles in vivo, related to the two latter sets of factors.

II. Objectives for the reporting period:

The changing with age of the respiratory tract geometry and of the ventilation, is usually thought to result in changes of particle deposition values. An accurate estimate of particle deposition in the growing airways is of special interest not only for dosimetry in children, but also for a reliable assessment of accumulated doses during a whole lifespan.

In order to establish the validity of various mathematical models, available experimental data are needed.

With the agreement of the Ethical Committee of the hospital Pitié-Salpétrière, total deposition by mouth-breathing, and nasal inspiratory deposition have been measured in healthy volunteers, adults and children.

The ventilation was controlled, and the tidal volume (V_T) and frequency (f_R) selected, according to age, body size and sex to standardize the physiological parameters at rest and at exercise.

III. Progress achieved:

Methodology

1. The aerosol generation-exposure system included principally (fig. 1) :

- a generator of monodisperse polystyrene beads (aerodynamic diameter : 1.1, 1.9, 2.6 and 3.05 µm) ;

- a controlled breathing device, with one-way valves, fleisch tubes, volume integrating system, and visual and auditive signals ;

- an aerodynamic particle sizer (APS), and concentration measuring device, based on the laser velocimetry principle.

2. Healthy volunteers, adult non smokers and children, have been explored by clinical examination and respiratory function testing :

- spirometric measurement of vital capacity (V.C.), total lung capacity (T.L.C.), residual volume (R.V.), functional residual capacity (F.R.C.) and forced expired volume in 1 s. (F.E.V.1) ;

- anterior rhinomanometric determination of nasal resistances, as a function of nasal flow-rates.

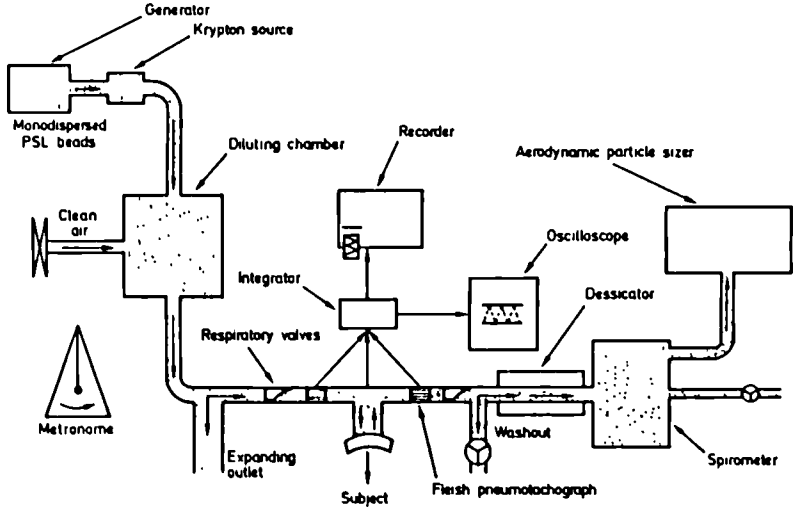


Fig. 1 : Aerosol generation-exposure system

3. Experimental procedure : after calibration of flow-rates, volumes, generated aerosol sizes and concentrations, particle deposition was measured in the subject's airways.

- during mouth-breathing, with a nose-clip ;

- during nose-breathing, through a nose-mask with the mouth shut. The deposition (D) of inert particles in the airways, can be measured by comparing their concentrations in the inhaled (Ci) and expired (Ce) aerosols ;

- deposition by mouth-breathing :

$$Dm = 1 - Cem/Ci$$

- déposition by nose-breathing :

$$Dn = 1 - Cen/Ci$$

(usually Dn > Dm)

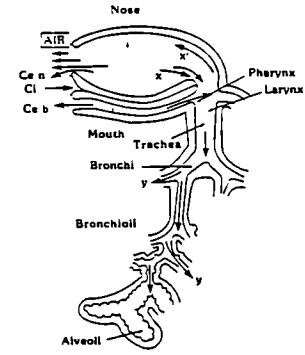


Fig. 2 : **Regional deposition in the airways**

Assuming that Dm is equal to the total sub-nasal deposition, y, (the mouth having a very low resistance) and that x and x' are the equal nose deposited fractions at inspiration and at expiration (fig. 2) the "nasal particle filtering efficiency" is given by :

$$x = 1 - \sqrt{Cen/Cem}$$

Results

1. Total deposition of particles by mouth-breathing

A group of 10 adults (5 men, 5 women) and 40 children aged 5 to 15 years, were asked to breathe at various levels of ventilation : the standard values were choosen from table 1.

Globally, in adults, total airway deposition increased with particle size, and with increasing ventilation. In children, although the data showed a high variation coefficient, the deposition was closed to the adult's, probably because of the ventilation scaling for age, sex and body size. However, at exercise, children's ventilation rates were not increased as much as the adult's, and the deposition became higher with progressing age.

Age y	Resting (sitting-awake) f_R min^{-1}	TV l	$\dot{V}E$ l·min^{-1}	Light exercise f_R min^{-1}	TV l	$\dot{V}E$ l·min^{-1}	Heavy exercise f_R min^{-1}	TV l	$\dot{V}E$ l·min^{-1}
6	23	0.250	5.75	38	0.290	11.0	50	0.370	18.5
8	23	0.280	6.0	37	0.460	17.0	48	0.580	28
10 M	20	0.340	6.80	32	0.580	18.6	44	0.840	37
F							46	0.670	31
12 M	18	0.430	7.75	27	0.760	20.5	38	1.06	40.3
F	19	0.340	6.45	30	0.630	18.9	44	0.820	36
14 M	15	0.540	8.1	25	0.930	23.2	37	1.22	45
F	18	0.370	6.7	26	0.770	20	42	1.0	42
16 M	14	0.620	8.7	23	1.10	25.3	36	1.40	50.4
F	17	0.410	6.9	24	0.900	21.6	37	1.215	45
18 M	13	0.770	10.0	21	1.25	26.2	34	1.5	51
F	15	0.470	7.05	22	1.0	22	36	1.25	45
30 M	12	0.750	9.0	20	1.25	25	26	1.92	50
(heavy F worker)	14	0.460	6.45	21	1.0	21	28	1.61	45

Table 1

f_R = frequency
TV = tidal volume
$\dot{V}E$ = minute ventilation

Physiological Values choosen from published experimental data.

2. Nasal particle filtering efficiency

A group of 10 adults and 20 children performed the deposition test by nose-breathing. Their nasal resistances, expressed as a function of inspiratory flow-rates decreased with progressing age (fig. 3).

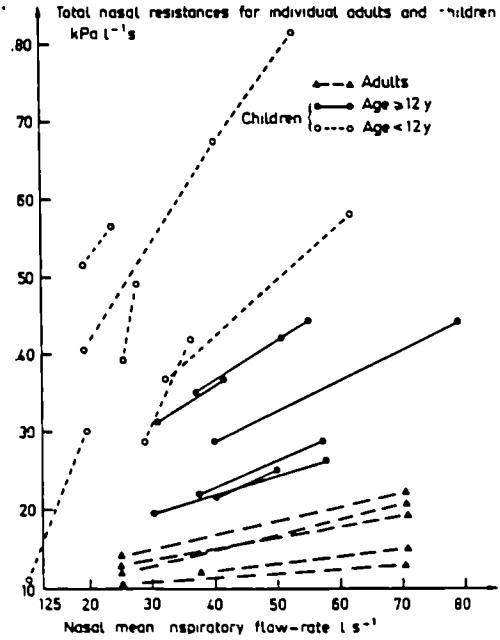


Fig. 3 : Nasal resistances versus flow-rates

- Nose deposition at inspiration did not follow the same pattern. Children's nasal particle filter was a little less efficient, especially for particles ⩾ 2 µm, when ventilation was standardized for age (fig. 4).

- Nasal deposition seemed better correlated with inspiratory flow-rates than with resistances.

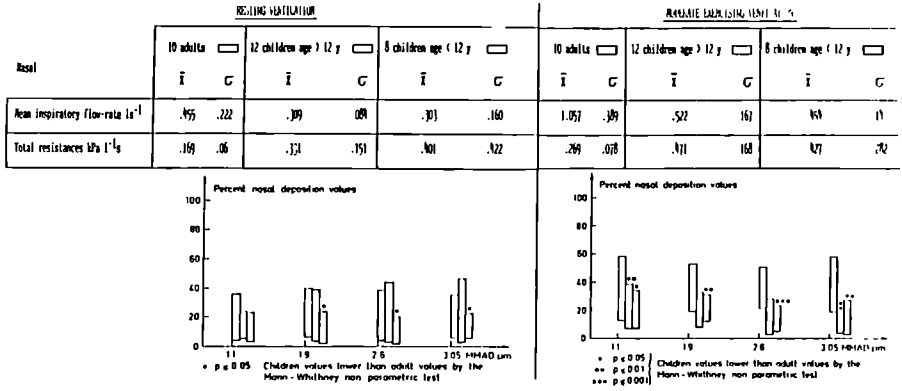


Fig. 4 : Nose deposition values in adults and children at two ventilation rates (at rest and at moderate exercise)

IV. Objectives for the next reporting period:

With the aim of making lung models applicable to various members of population, we propose to study the influence of :

1. Interracial variations

For example, compared to europeans, africans are said to have smaller lung volumes and lower nasal resistances.

2. Pulmonary pathology induced modifications

In two types of patients with :

- restrictive lung function (who have small lung volumes) ;
- obstructive lung function (who have low flow-rates and high airway resistances).

These conditions are thought to strongly influence the aerosol deposition.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. Service Central d'Explorations Fonctionnelles Respiratoires
   Hôpital La Salpétrière (Professeur A. TEILLAC)
   47, Boulevard de l'Hôpital - 75651 PARIS CEDEX 13 (France)

2. Institut für Biologie, Abteilung für Biophysikalische
   (Dr. W. STAHLHOFEN) Strahlenforschung
   Paul-Ehrlich-Strasse 15 u. 20
   D-6000 FRANKFURT/MAIN (R.F.A.)

3. Johns Hopkins University School of Hygiene and Public Health
   (Professor David L. SWIFT)
   615 North Wolfe Street - BALTIMORE Maryland (U.S.A.).

VI. Publications:

A.C. JAMES, M. ROY. Dosimetric lung models in "Age-related factors in radionuclide metabolism and dosimetry". G.B. GERBER, H. METIVIER, H. SMITH Ed. 1987 : 95-108. Martinus Nijhoff, DORDRECHT for C.E.C.

M. ROY, D.L. SWIFT, M.H. BECQUEMIN, A. GAHEM, A. BOUCHIKHI, D. RODRIGUE. Nasal resistances and particle nose deposition in healthy adults and children. Journal of Aerosol Medicine, 1988 Vol. 1, n° 3, p. 236.

A. BOUCHIKHI, M.H. BECQUEMIN, J. BIGNON, M. ROY, A. TEILLAC. Particle size study of nine metered dose inhalers, and their deposition probabilities in the airways Eur. Resp. J. 1988 - 1, 547-552.

M.H. BECQUEMIN, A. BOUCHIKHI, M. ROY, J. CARLES, A. TEILLAC. Total particle deposition in the normal respiratory tract : influence of ventilatory parameters. Journal of Aerosol Medicine, Vol. 1, n° 3, 1988, p. 191.

A. BOUCHIKHI, M.H. BECQUEMIN, C. HARPEY, M. ROY, A. TEILLAC. Fusafungine : Aerosol droplet features and total deposition in obstructive and healthy subjects' airways. Journal of Aerosol Medicine, Vol. 1, n° 3, 1988, p. 246.

M.H. BECQUEMIN, B. DAUTZENBERG, A. BOUCHIKHI, M. ROY, F. ANTOUN, A. TEILLAC. Optimization of Pentamidine aerosol administration for acute lung disease treatment. Journal of Aerosol Medicine, Vol. 1, n° 3, 1988, p. 244.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: B16-D-201-F

Université Paul Sabatier
Faculté de Médecine Purpan
Allées Jules Guesde, 37
F - 31073 Toulouse Cédex

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. H. Planel
Lab. de Radiobiol. & Biol. Spatiale
Université Paul Sabatier
Allées Jules Guesde, 37
F - 31073 Toulouse Cédex

Telephone number: 061-25.21.23

Title of the research contract:

Biochemical and biophysical studies on the effects of very low doses of ionizing radiation on cells.

List of projects:

1. Biochemical and biophysical studies on the effects of very low doses of ionizing radiation on cells.

Title of the project no.: B16 - D201F

Biochemical and biophysical studies on the effects of very low doses of radiation on cells.

Head(s) of project:

Pr. H. PLANEL - Laboratoire de Radiobiologie et Biologie spatiale - Faculté de Médecine - TOULOUSE -

Pr. F. STEINHAUSLER - Institüt für Biologie, biochemie un biophysik - SALZBURG -

Scientific staff:

F. CROUTE, Y. GAUBIN and B. PIANEZZI (Toulouse University)

B. RUBEL, M. MEISS, C. ATZMULLER and M. HUBER (Salzburg University)

I. Objectives of the project:

Contrary to high doses of ionizing radiation, effects of very low doses, less than 0.1 Gy, are not well known. However, previous experiments have shown that very low dose rates of chronic irradiation can stimulate the proliferative activity of Paramecia and Cyanobacteria (PLANEL et al. Health Physics, 1987). We intend to investigate, in this programme, the effects of very low doses of gamma rays on human cells cultivated in vitro.

II. Objectives for the reporting period:

1) Based on radiation standards, we intend to investigate the effects of 0.05 Gy in human dermal fibroblasts, given for a short period of time. Investigations will be carried out also in human keratinocytes : indeed possible changes induced by radiations on dermic cells in living organisms could appear through the release of epidermal cytokines produced by the keratinocytes (investigations carried out in Toulouse laboratory).

2) New investigations will be performed in order to confirm the stimulating effect of a long-term irradiation, at a very low dose rate, using a great number of cultures in view of statistical analysis of results (investigations carried out at Salzburg Institute.

III. Progress achieved:

1.- METHODOLOGY

Cell cultures : Human dermal fibroblasts were cultivated in 10% calf serum supplemented MEM. A spontaneously transformed BALB/c derived keratinocyte line (PAM 212) was also used as a source of cytokine inter-leukine 1; confluent monolayers PAM 212 were incubated in serum free MEM at 37°C and irradiated for 2 or 3 days only (due to nutritional conditions). Supernatants were harvested after irradiation, filters sterilized and stored at -20°C when not immediatly used. Control supernatants were obtained from non-irradiated cultures.

Irradiation facilities : For chronic irradiation, cell cultures were exposed for several months to a planar source containing Thorium nitrate at a dose rate of 0.06 mGy/day. For short term irradiation (8 days maximum), cultures were exposed to a ^{60}Co source, at a dose rate of 6.25 mGy/day at the culture level.

Technics

a) For chronic irradiation, cells in the culture flasks were suspended and equally distributed in a matrix of 8 x 11 wells of titer plates, where they continued to proliferate for several days. The same procedure with the same amount of cells was carried out with the control cells.After reaching the transition from the log-phase-growth to the plateau-phase-growth, the cultures were prepared for photometric growth measurements as follows : after removal of the medium, the cells are stained with a neutral red solution for about 15 min., washed and inserted into the photometer. A light beam with a wave length of 492 nm passes through each single well in the whole sample; the net-absorption of the light by the sample is detected and recorded. Because the absorption in each well increases with the number of the stained cells, the relative cell growth can be determined by the absorption factor of each well. The absorption factors of 88 single wells are summarized in (geometrical) mean and (geometrical) standard deviation. The comparison between data groups is carried out using the two-sized distribution-free U-test with a level of significance of $\alpha = 0.05$. Two titer plates with irradiated cells and two plates for control cells were measured in each serie.

b) For short time irradiation, the cell populations were measured with a cell coulter. Biochemical investigations were performed in cells exposed to a maximal dose of 0.05 Gy.

2.- RESULTS

a) Chronic irradiation : as shown in Figure 1, skin cell cultures pre-irradiated with 7.5 mGy for 118 days and with 9.3 mGy for 155 days show statistically significant increased cell growth.

b) Short time irradiation : the results of 5 experiments are exposed in Tables 1 and 2. Cell populations were determined every day from the 3th to the 8th day. Irradiation did not induce significant changes in cell populations, protein content, catalase and G6PD activities (Table 1). However, the pyruvate kinase activity appeared inhibited in the early exponential growth phase (differences are significant $p \leq 0.05$).
An influence of irradiation on the secretion of IL1 (or cytokine) was investigated in keratinocytes irradiated for 2 or 3 days. As shown in Table 2, the growth of fibroblasts cultivated in medium after addition of supernatant from irradiated cultures remained unchanged.

3. - DISCUSSION

A radiation hormesis appears when human cells were submitted for several months to a chronic irradiation at a very low dose rate, maximal dose beeing 9.3 mGy. This data confirms previous experiments (CROUTE et al. 1986). However, when cells were exposed to a short term irradiation, in spite of higher levels of dose, ie, 0.05 Gy, the irradiation did not induce changes in cell growth rate. On the other hand, it is well known that keratinocytes produce cytokines which stimulate fibroblasts growth, collagene and elastase synthesis (BREATHNACH et al. 1988, CROUTE et al. 1988). It can be concluded that possible indirect effect of radiation on skin could be produced through changes in cytokine production. When exposure to UV can reduce the cytokine synthesis (SCHWARZ et al. 1987), our investigations show that, in our experimental conditions, gamma rays appear to have no effect. However, the same investigations demonstrate that cell metabolism can be affected as shown by changes in pyruvate kinase, ie, in an enzyme involved in the glycolysis pathways.

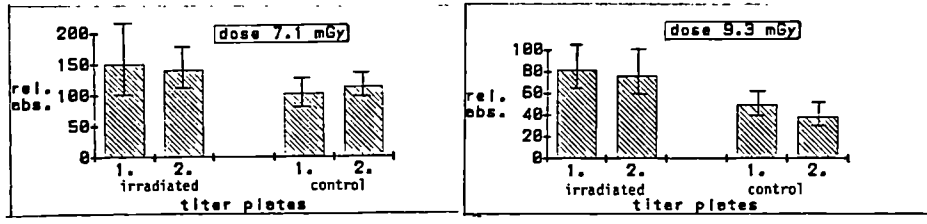


Figure 1

Parameter		n^th day	5	6	7	8
Cell popul./cult.	C	1.214044 ± 45 298	1.429 027 ± 73 657	2 433 125 ± 58 080	2 628 173 ± 36 340	2 697 619 ± 45 285
	I	1.186 333 ± 66 289	1.399 676 ± 53.544	2 310 000 ± 128 108	2 641 898 ± 91 683	2 706 472 ± 73 088
Protein g/10^6 cells.	C	227.6 ± 19.6	228.3 ± 5.5	225.5 ± 5.3	247.3 ± 3.7	237.5 ± 10.8
	I	216.0 ± 21.8	237.6 ± 9.8	224.3 ±12.5	251.3 ± 6.8	237.5 ± 5.5
Catalase I U	C	9.43 ± 0.98	13.74 ± 0.62	-	10.75 ± 0.82	9.88 ± 0.54
	I	10.29 ± 1.65	13.88 ± 0.58	-	10.77 ± 0.48	9.82 ± 0.81
GB PD I U	C	-	74.36 ± 1.83	-	80.52 ± 3.46	73.61 ± 4.7
	I	-	67.77 ± 3.88	-	78.78 ± 7.65	69.01 ± 1.8
Pyr. Kinase I U	C	355.08 ± 24.42	564.3 ± 41.74	634.17 ± 38.72	810.5 ± 39.9	807.38 ± 62.95
	I	272 ± 35.55	426 ± 75.8	514.89 ± 71.88	776.7 ± 107.84	680.00 ± 33.74

Table 1 (I.U : International Unit).

Duration keratinocyte irradiation		2 Days		3 Days	
		C	I	C	I
Fibroblast populations / Culture	3th day	321 081 ± 6724	325 818 ± 8435	223 604 ± 6146	228 087 ± 8418
	4th day	801 405 ± 4370	484 054 ± 11268	354 250 ± 26260	369 365 ± 17574
	5th day	705 406 ± 21710	727 425 ± 12981	501 264 ± 31488	510 569 ± 34218

Table 2

IV. Objectives for the next reporting period:

We intend :

- to investigate the effects of very low doses on cell metabolism, in particular on enzymes involved in carbohydrate metabolism. Using the same dose (0.05 Gy), the glyceraldehyde-3-phosphate dehydrogenase, the pyruvate-kinase, the pyruvate-dehydrogenase and ATP will be determined (after cell count and protein content measurements).

- to investigate, in parallel, a possible effect of very low doses on cell membrane. This effect will be investigated using the method of gel electrophoresis introduced recently in the Institute of Biophysics of Salzburg University. Cells will be exposed to short-term or chronic irradiations, in both cases at very low doses of gamma rays.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-236-D

Gesellschaft für Strahlen- und
Umweltforschung mbH
Ingolstädter Landstrasse 1
D - 8042 Neuherberg

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. W. Pohlit
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Telephone number: 069-6303311/319

Title of the research contract:

RBE-values of monoenergetic electrons in the range of several 100 eV to 10 keV for cell transformation.

List of projects:

RBE-values of monoenergetic electrons in the range of several 100 eV to 10 keV for cell transformations.

Title of the project no.: 1

RBE-values of monoenergetic electrons in the range of several 100 eV to 10 keV for cell transformation

Head(s) of project:

Priv.Doz.Dr.D.Frankenberg

Scientific staff:

Priv.Doz.Dr.D.Frankenberg
Dr.M.Frankenberg-Schwager

I. Objectives of the project:

RBE-values of electrons of definite energies will be determined for cell transformation using the immortalized mouse embryo fibroblast cell line C3H 10T1/2. Monoenergetic electrons are produced within cells by irradiation with characteristic ultrasoft X-rays which are generated by bombarding suitable targets with accelerated protons. The radiations used are ^{60}Co gammarays as refererence radiation and Al_K (1.5 keV) and C_K (0.278 eV) characteristic X-rays.

II. Objectives for the reporting period:

During the reporting period the experimental methods for investigation of cell transformation in vitro are established in our laboratory. Inactivation and first experiments of cell transformation are performed. Special methods for exposure of cells by ultrasoft X-rays are developed.

III. Progress achieved:

Methodology

Cells of passage 14 were cultured in 75 cm^2 flasks and incubated in a humified gas atmosphere (94 % air, 6 % CO_2) at 37°C. The plating efficiency of control cultures was 20 to 30 per cent. 24 hours before irradiation, cells were plated in 25 cm^3 flasks. Cells were exposed to ^{60}Co gammarays in 25 cm^2 flasks at a cell density of about 10^4 cm^{-2}. After radiation exposure, cells were subcultured in 25 cm^2 flasks with about 70 viable cells per flask for inactivation studies and with about 250 viable cells per flask for transformation studies. For cell survival,clones were counted after 2 weeks. For cell transformation, foci of type 2 and 3 were scored as transformants after 6 weeks.

Results and Discussion

In figure 1 is shown the survival of C3H 10T1/2 cells after irradiation with ^{60}Co gammarays at a dose rate of 0.7 Gy per minute. The survival curve is in excellent agreement with that obtained by Hieber et al. (1988) using the same cell line. As preliminary results table 1 presents the transformation frequencies per surviving cells after ^{60}Co gamma irradiation for 3 and 5 Gys. The survival curve in figure 1 was used to correct for the cells killed by the irradiation. The data are in agreement with the findings of Miller et al (1979) and Hieber et al (1988).

Table 1

Preliminary transformation rates after irradiation with ^{60}Co gammarays for 3 and 5 Gys at a dose rate of 0.7 Gy per minute.

D/Gy	plating efficiency	survivors	trans-formants	transformants per 10^4 survivors
3	0.14	6.4×10^4	21	3.3
5	0.03	2.1×10^4	20	9.5

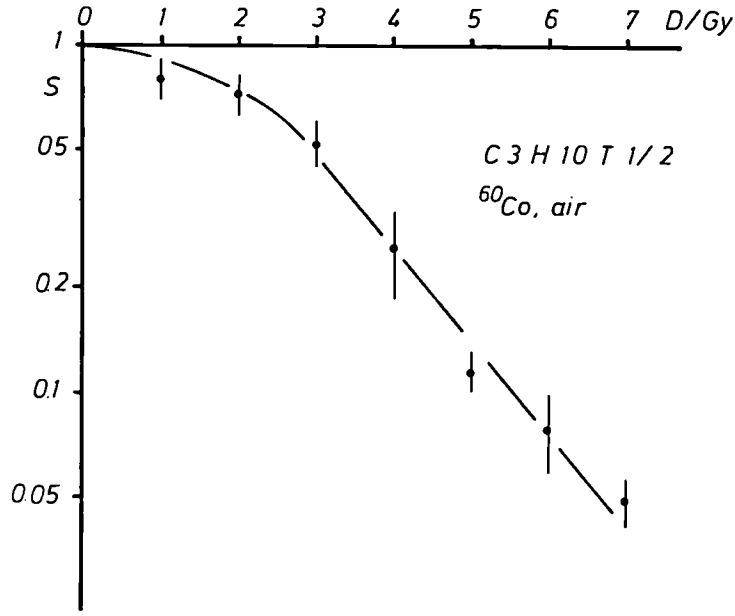


Figure 1

Inactivation S of C3H 10T1/2 cells by ^{60}Co gamma rays at a dose rate of 0.7 Gy/min

References

Hieber, L., Ponsel, G., Roos, H., Fenn, F., Fromke, E. and Kellerer, A.M., Int.J.Radiat.Biol. 52, 859-870 (1987)

Miller, R.C., Hall,E.J., Rossi, H.H., Proc.Natl. Acad.Sci.(USA), 76, 5755-5758 (1979)

IV. Objectives for the next reporting period:

Inactivation and tranformation frequencies of C3H 10T1/2 cells will be determined after irradiation with ^{60}Co gammarays, Al_K and C_K characteristic X-rays. Using the results obtained with ^{60}Co gammarays the RBE-values of Al_K and C_K characteristic X-rays are evaluated for cell inactivation and cell transformation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr.L.Hieber, Institut für Medizinische Strahlenkunde der Universität Würzburg, Versbacher Str. 5, D-8700 Würzburg

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-102-UK

United Kingdom Atomic Energy
Authority
Atomic Energy Establishment
Winfrith, Dorchester
GB - Dorset DT2 8DH

Head(s) of research team(s) [name(s) and address(es)]:

Dr. D. Ramsden
Radiological & Safety Division
Atomic Energy Establishment
Winfrith, Dorchester
GB - Dorset DT2 8DH

Telephone number: 0305-63111

Title of the research contract:

Plutonium exposures in man. Direct monitoring of the lung, reassessment of the ICRP lung model and 'solubility' studies.

List of projects:

1. The direct determination of the distribution of activity within the lung.
2. Lung models.
3. In-vitro solubility studies.

Title of the project no.: 1

The direct determination of the distribution of activity within the lung

Head(s) of project:

D Ramsden
P P Foster

Scientific staff:

K P Kingman (½ year)

I. Objectives of the project:

The unknown distribution of particulate material in the lung, following accidental intakes of the transuranic compounds, and the changes in distribution that occur with time following such intakes are identified as the major sources of uncertainty in assessment programmes based on direct monitoring of the human thorax with external detector arrays. This Project is aimed at quantifying and reducing these uncertainties and is intended to be applied at the low contamination levels encountered in routine lung monitoring programmes. The approach is one of determining the most probable distribution pattern in each individual by means of statistical analysis of the monitor data and by computer modelling of the individual chest structure.

II. Objectives for the reporting period:

Confirmation of previous methodology using an alternative array.

Measurement programme cn man.

Publication of final report on the project.

III. Progress achieved:

This project was planned to be completed in 1988 with a publication of the final report. The loss of responsible scientist early in the year meant that this objective was not met. As a consequence of the loss of the research scientist, effort was diverted to project 2, with consequential enhanced progress of that project. The equipment and some of the methodology developed previously under project 1 was then used routinely in 1988 for the assay of plutonium in lung of the local workforce. The total number of measurements made under this programme was 211. Some modifications to the hardware and 'fine tuning' of the software proved to be necessary during this programme. The overall result indicated that the system performed well, achieving the main objective of lowering measurement uncertainties - even without applying the fully developed computer modelling programme. All the results obtained reflected the very low levels, or absence, of plutonium materials within the lung of the workforce. The distribution of results is given in the Figure. The results are presented as a histogram, together with a fitted normal distribution, of assessed total alpha activity in the lung. Total alpha activity consists of the plutonium isotopes 238, 239, 240 together with Am 241 where the relative proportions are determined from air samples or from faecal measurements. Average americium contents of the contaminant are of the order of 30% by alpha and the results are the weighted means of X-ray and γ ray determinations. The width of the distribution (σ) of 33 Bq should be compared with a similar distribution reported in 1980 of 133 Bq (IRPA Jerusalem Vol II pp 119) demonstrating the improvements made over the course of this project.

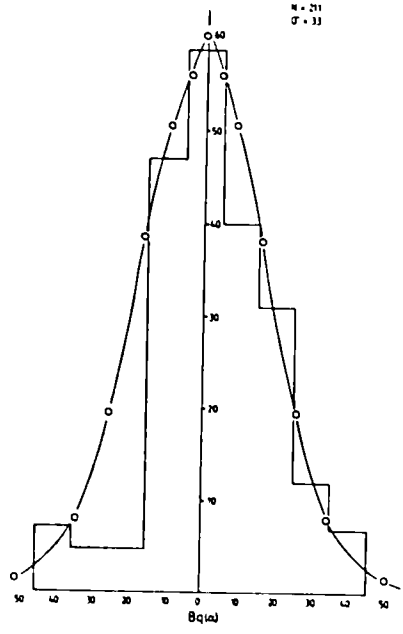


Fig. Histogram of 'plutonium in lung' data

IV. Objectives for the next reporting period:

The final report on this project

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

The work is supported under the UKAEA Radiological Protection Research Core Programme and as such the programme is discussed with other workers in the field within UK.

VI. Publications:

None

Title of the project no.: 2

Lung Models

Head(s) of project:

D Ramsden
P P Foster

Scientific staff:

I Pearman

I. Objectives of the project:

The overall objective of the project is to obtain data on man for use in developing models of lung retention and clearance. The models are then extended to describe urinary excretion, specifically for compounds of plutonium. Compartmental models of the respiratory tract, essentially modifications the ICRP methodology, have been developed and combined with urinary excretion models. The parameters in the models are chosen to match observations in man with accidentally inhaled low levels of plutonium oxide dusts.

The other source of data from man is obtained from human volunteer studies. Winfrith participated in the EULEP supported Joint European interspecies intercomparison of lung clearance oxide (Co 57).

II. Objectives for the reporting period:

a) Completion of the measurements of Co57 in human volunteers

b) Report for the joint EULEP study

c) Report of measurements after completion of the EULEP study

d) Report comparing observed levels of plutonium in urine with levels predicted by various metabolic models.

The fourth objective was added during the reporting period as effort was diverted from project 1.

III. Progress achieved:

a) Co 57 in human volunteers

The residual Co 57 in the lungs of human volunteers has been measured up to 1000 days after intake. The levels are now below the limit of detection. The EULEP study was completed at some 200 days after intake. Faecal and urinary clearances were below limit of detection at about 600 days.

Figure 1 to 3 summarise the measurements, expressing the data as the fractions of initially deposited material corrected for radioactive decay. They present the average value for the four volunteers. The difference in behaviour of the two particle sizes employed (2.7 and 1.4 µm) are only noticable in the first few weeks after intake and the results are combined in the figures which illustrate late behaviour. The curves are compared to ICRP retention and urinary excretion curves for Class W and Class Y behaviour of the average particle size, corrected for mouth breathing.

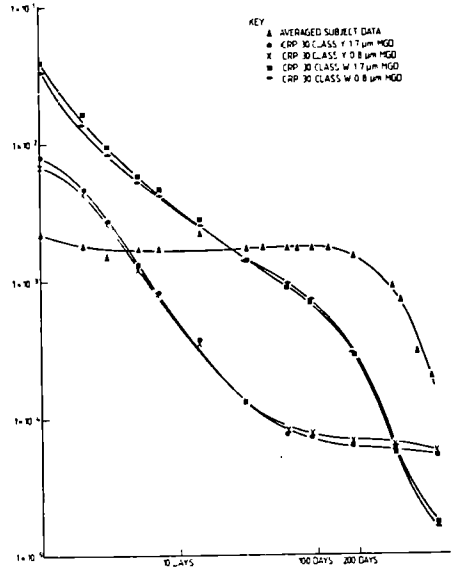


Fig. Daily Urine Excretion as a fraction of deposition

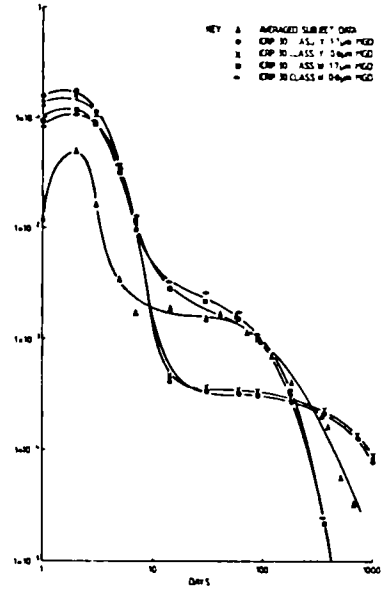


Fig. Daily Faecal Excretion as a fraction of deposition

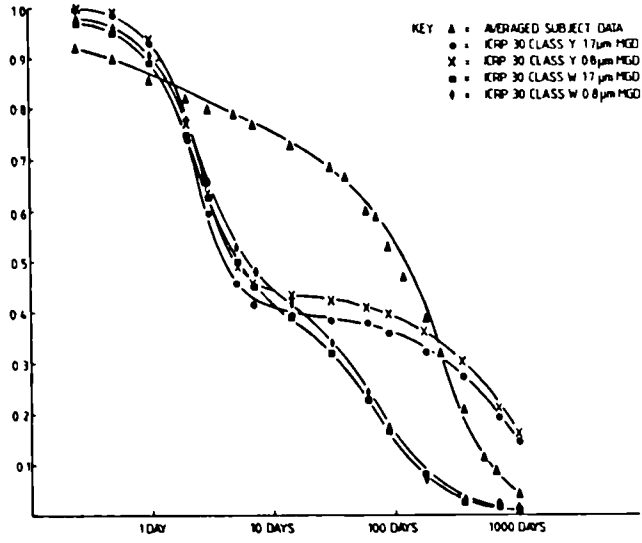


Fig. Lung retention as a fraction of deposition

The lung retention curve is now following Class W clearance (τ ½ days) with no evidence of a long term lymph mode retention. The initial deposition and clearance are consistent with minimal deposition in the upper respiratory tract and with no short half life components. Similar comments can be made regarding the urinary excretion pattern from these subjects when again the curve is consistent with type W behaviour and with the absence of upper respiratory tract deposition or short half life components.

The joint report of the interspecies intercomparison will be published in March 1989 (J of Aerosol Source). The data for man is presented in terms of mechanical transfer faction and solubilised fractions and typical values for these are:-

Mechanical transfer fraction	0.05% per day
Solubilised fraction	0.4 % per day

The data between days 200 and 1300 has been submitted and accepted for presentation at the SRP International Symposium in Malvern June 1989.

b) Metabolic Models

The models for lung behaviour and for urinary excretions, developed under this project have been published previously. These specifically quantify and describe the behaviour of inhaled mixed oxide dusts. The fit of the lung model with observations on man was good and was used as the basis of the justification and use of site specific ALIs in a paper at the CEC Workshop on inhaled actinides (Versailles 1988). The fit of the urinary excretion models were somewhat variable and should be expected to quantitatively describe inhalations of all plutonium compounds. Through the auspices of the UK Internal Radiations Dosimetry Group, data sets of typical excretion levels following different exposure patterns to different plutonium materials are being established. Initial work on comparing the performance of different excretion models is completed. The model developed under this project was tested on these data. The preliminary result, yet to be verified, indicate that, for a wide range of material type, exposure patterns and sampling times, the different models agree to within 50% and that much of this variation can be attributed to scatter in the data and to the statistical treatment.

Discussion

The objectives have been met and somewhat extended. The work is largely complete - save for the preparation of the final report. The data from the project will then need to be combined with the data for project 3 - the in-vitro study of mixed oxide dusts.

IV. Objectives for the next reporting period:

a) Final report of Co57 in man

b) Report on comparison of urinary excretion models for plutonium in man.

c) A combined final report on project 2 and 3.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Co57

GSF	FRG
NRPB	Chilton UK
CEA	Bruyeres le-Chatel F
MRC	Harwell UK
IGT/KFK	Karlsruhe FRG
AERE	Harwell UK
VIC	Karlsruhe FRG

Modelling Work

Internal Radiations Dosimetry Group UK

VI. Publications:

Justification, calculation and use of Site Specific ALI

D Ramsden, P P Foster CEC Workshop on Occupational Exposure to inhaled actinides Versailles 1988

Title of the project no.: 3

In-vitro Solubility Studies

Head(s) of project:

D Ramsden
P P Foster

Scientific staff:

I Pearman
M E Bains

I. Objectives of the project:

The 'solubility' in man of dusts from mixed plutonium/uranium oxide fuels determines the validity of urine monitoring as a method of assessing systemic burdens and intakes. This project studies the long term 'in-vitro' solubility of suspended particles from such fuels in laboratory rigs simulating the human lung under careful, controlled conditions. The main difference from other studies was in the time-scale of the experiment being three years, a period comparable with the long term solubility components in current lung models.

Data from the project will provide parameters for project 2.

II. Objectives for the reporting period:

a) Completion of analysis on residual activity within experimental rigs.

b) Preparation of the final report

c) Comparison of data with parameters used in project 2.

III. Progress achieved:

Methodology

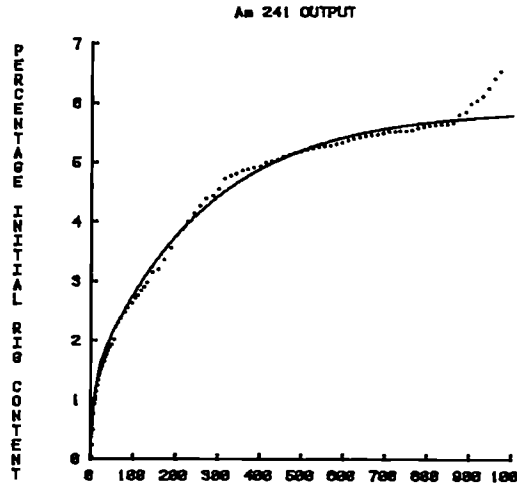
The methodology of the experimental phase was described in last year's report. The patterns of 'solubilisation' for the three rigs were determined during the course of the study for the radionuclides Am 241, Uranium 234 and 235, Plutonium 239 and 241. The patterns are analysed as a series of exponential terms for each rig and each radionuclide. Best fitting techniques are used. The exponential terms are then to be compared to those published for urinary excretion of plutonium in man and a 'standardised' set of terms applied to the modelling projects (Project 2).

Results

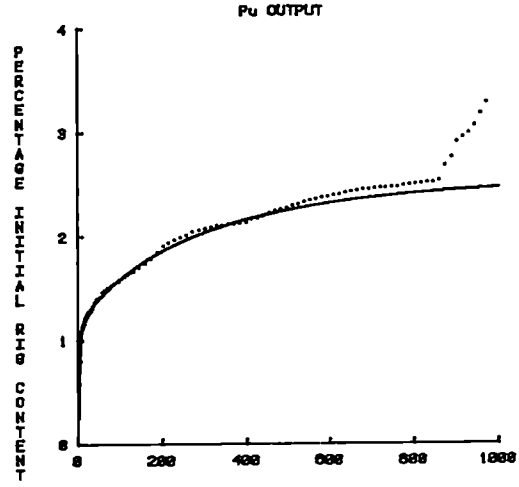
The experimental data is now complete with the exception of some samples resulting from the dismantling of the rigs. The delay was due to a major refurbishment of the analytical laboratories. The outstanding samples will affect the absolute magnitude of the amplitudes of the exponential terms in the data analysis but will not affect the exponents nor the relative amplitudes. Graphs 1 and 2 show typical fits of the data to a sum of exponential terms, for the radionuclides Am 241 and Pu 239. The data is fitted to the sum of three terms, with half lives varying from 60 to 14000 days. Shorter term components have been discussed in previous reports.

The values of the half lives obtained, from both plutonium and Americium are comparable with those published by Durbin for the the excretion pattern of plutonium in urine. However the amplitudes, both absolute and relative, of these components are not comparable to those of Durbin. In particular we observe that the long term component (4000 day half life) predominates.

The total amplitude, ie the percentage of activity eventually solubilised, agrees with ICRP Class Y behaviour at between 2% and 5% of the actual activity. Further analyses are in progress.



Rig No 1 Cumulative Amercium solubilised fraction as percentage of initial contents



Rig No 1 Plutonium 239

Discussion

The analysis of all the data is still not complete but interim results are showing that the time scales (half lives) and the total fractions available for 'solubilisation' are comparable with current models of the behaviour of plutonium in man. The relative magnitudes of the different exponents differ markedly from published data and the final step of the project is to combine this data with the modelling projects. It is anticipated that this combined approach will show that plutonium assessments based on long term urinary excretion will not differ markedly but that assessments based on short and medium term excretion will produce lower estimates of systemic uptake than assessments using on current models of plutonium dosimetry.

IV. Objectives for the next reporting period:

(a) Publication of final report on this project

(b) Combination of projects 2 and 3

(c) Publication of final report on combined subjects

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Contacts with NRPB

VI. Publications:

(a) None

(b) Quarterly progress reports to Radiological Protection Research Core Programme Committee (UK)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-D-178-B

Université Libre de Bruxelles
avenue F.D. Roosevelt, 50
B - 1050 Bruxelles

Head(s) of research team(s) [name(s) and address(es)]:

Prof. J. Rommelaere
Dépt. de Biologie Moléculaire
Université Libre de Bruxelles
rue des Chevaux, 67
B - 1640 Bruxelles

Telephone number: 02-358.35.30

Title of the research contract:

Cooperation between radiation and oncogenes in malignant transformation of mammalian cells.

List of projects:

1. Cooperation between radiation and oncogenes in malignant transformation of mammalian cells.

Title of the project no.: 1. Cooperation between radiation and oncogenes in malignant transformation of mammalian cells

Head(s) of project: J. ROMMELAERE

Scientific staff: J. CORNELIS
G. HILGERS
B. AVALOSSE
S. BARRIJAL
Y.Q. CHEN

I. Objectives of the project:

This project has four main objectives :

a. To characterize phenotypic alterations which are induced by radiation in human fibroblasts and epithelial cells and which commit them into malignant progression.
b. To develop a quantitative assay for monitoring the transforming effects of ionizing radiation on human epithelial cells.
c. To identify synergistic actions between radiation and oncogenes for malignant transformation of human and murine cells.
d. To study cellular mechanisms underlying the cancer-proneness and radiohypersensitivity associated with the human syndrome ataxia telangiectasia.

II. Objectives for the reporting period:

a. Subproject a was an attempt at identifying specific nuclear proteins whose expression is up- or down-modulated in human fibroblasts transformed by ionizing radiation.
b. Subproject b aimed at developing a short-term quantitative assay for the measurement of radioinduced alterations in the requirements of human epithelial cells for growth and differentiation modulators.
c. Subproject c dealt with the phenomenological and molecular analysis of transient conditional responses induced by radiation in human fibroblasts from normal individuals and patients with the radiohypersensitivity syndrome ataxia telangiectasia.

III. Progress achieved:

Subproject a. Identification of molecular changes accompanying radiation-induced transformation of human fibroblasts by means of parvoviral DNA probes.

- Advantage was taken of the enhanced replication and expression of parvoviral DNA in gamma ray-transformed human fibroblasts, to fish out candidate transformation-modulated cellular proteins by means of their binding to the parvoviral genome.
- Human fibroblasts immortalized and transformed by ionizing radiation differ from normal parental strains by their much greater susceptibility to the lytic action of the autonomous parvoviruses MVMp and H-1. Such an hypersensitivity of transformed cells to parvoviral attack correlates with a striking increase in their ability to synthesize viral DNA, messenger RNAs and (non)structural proteins, as determined by Southern, Nothern and immunoprecipitation analyses, respectively. These observations indicate that cellular changes accompanying radioinduced transformation can be revealed by their effects on infecting parvoviruses.

This prompted us to use the parvoviral genome as a probe to identify cellular DNA-binding proteins that may take part in the replication and/or expression of viral DNA and be present in different quantities and/or qualities in radiotransformed versus normal human cells. Using the South-Western technique, normal and gamma ray-transformed human fibroblasts were compared for their content in proteins that are able to trap parvoviral DNA. Preliminary results suggest that radiation-induced transformation is indeed accompanied by several changes in parvoviral DNA-binding cellular proteins. In particular, a nuclear protein that specifically recognizes terminal parvoviral DNA restriction fragments, appears to be either lacking or blocked in the transformants. The expression of the latter transformation-sensitive nuclear factor segregates with cell resistance to parvoviruses in hybrids between normal human fibroblasts (non-permissive) and transformed mouse cells (permissive) that randomly loose human chromosomes.

- These results are intriguing, given the accumulating evidence that neoplastic progression involves not only the activation of positive determinants but also the inactivation of negative ones. Indeed, our data raise the possibility that radioinduced transformation may overcome a cellular suppressor function that prevents parvoviral DNA replication and/or expression by interacting with the viral genome. The transformation step responsible for this overcome seems to be early as the transformed cells tested are not yet tumorigenic upon implantation in nude mice.

Subproject b. In vitro transformation of human keratinocytes by radiation.

- As reported previously, a culture system was set up for the clonal growth of normal human keratinocytes in a chemically defined medium containing specific growth factors. By varying the concentrations of cations and growth factors, we determined conditions allowing the proliferation of a spontaneously established line of human keratinocytes while precluding the growth of normal human epidermal cells.
- The use of the latter conditions to select transformed variants from a population of normal human epithelial cells was ascertained by performing reconstruction experiments in which normal and immortalized keratinocytes were mixed in different proportions. The colony-forming ability of the latter cultures plated under selective growth conditions proved to be proportional to their content in preexisting transformed cells.
- This system is now being used with the object of detecting radiation-induced phenotypic alterations that may contribute to neoplastic conversion of human epithelial cells. Cultures of normal human keratinocytes exposed

to a single dose of X-rays (30% cell survival) gave rise to colonies developing under selective conditions that totally suppressed the clonogenicity of unirradiated cells. The characterization of residual colonies as well as dose-response experiments are presently in progress in order to validate this assay for the quantitation of transforming effects of radiation on human cells.

Subproject c. Transient conditional responses to radiation in cells from normal individuals and ataxia telangiectasia (AT) patients.
- Radiosensitive cells from patients with ataxia telangiectasia (AT) were further studied for their proficiency in two putative eukaryotic SOS-like responses, enhanced reactivation (ER) and enhanced mutagenesis (EM) of incoming viruses. As reported previously, a line of AT fibroblasts (AT5BIVA) could not be induced to express ER of damaged parvovirus H-1, a single-stranded DNA virus, upon UV- or X-irradiation, whereas normal human cells were proficient in this response. Morever, preliminary evidence suggested that AT5BIVA cells may also be deficient in the radioinduction of the mutagenic EM response. The impairment of EM in AT cells was now confirmed, using damaged viral probes containing either single-stranded (H-1 virus) or double-stranded (Herpes simplex virus 1, HSV-1) DNA. In contrast, dose response and time course experiments revealed normal levels of ER of HSV-1 in X- or UV-irradiated AT5BIVA cells. Altogether, these data suggest that AT cells are altered for a process which plays a minor role in ER of a double-stranded DNA virus but which contributes to the survival of a single-stranded DNA virus as well as to the mutagenesis of both types of damaged-viruses. This deficiency seems to be associated with the radioresistant DNA synthesis typical of AT cells. These data may be explained by an impaired capacity of AT cells for replicating damaged viral DNA. Alternatively, irradiated AT cells may fail to produce signals that activate the latter process. It should be stated, however, that AT cells were as proficient as normal fibroblasts in the radioinduction of a series of specific messenger RNAs that were detected by Nothern blot analysis, using cDNA probes available in our laboratory.
- On the basis of the reported protective effect of interferons (IFN) against cell killing by radiation, IFNα was tested for its influence upon the ER response. Radiation and IFNα acted synergistically for ER induction in normal human fibroblasts. An attempt was made at investigating the latter phenomenon at the molecular level by measuring a series of reference messenger RNAs in cells exposed, or not, to radiation and/or IFNα . Preliminary results suggest that the steady state level of specific IFN-induced mRNAs was increased in irradiated cells. This effect appears to concern, in particular, transiently expressed mRNAs, raising the possibility that part of the inductive action of radiation may be ascribed to the stabilization of short-lived cellular messenger RNAs.

IV. Objectives for the next reporting period:

a. Search for protein markers of radiation-induced transformation of human fibroblasts : analysis of transformation-sensitive cellular proteins that specifically bind to parvoviral DNA (biochemical properties,DNA responsive elements, ubiquity, chromosomal mapping).
b. Validation of an in vitro assay for radiation-induced transformation of human epithelial cells : determination of dose-response curves and characterization of transformed colonies.
c. Study of transient conditional responses to radiation : investigation of synergistic inducing effects of radiation and interferon; molecular analysis of gene expression at the mRNA level.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Laboratory of Molecular Oncology, Institut Pasteur de Lille, 5019 Lille, France
- Division of Differentiation and Carcinogenesis in vitro, German Cancer Research Center, Deutsches Krebsforschungszentrum, D 6900 Heidelberg (N.E. Fusenig)
- Sylvius Laboratorium, Rijksuniversiteit, Leiden 2333 AL, The Netherlands (A. van der Eb)
- Department of Pathology, Kawasaki Medical School, Kurashiki, Japan (M. Namba)

VI. Publications: 1988

a. Directly relevant to the programme

- Hilgers, G., Abrahams, P.J., Schouten, R. Cornelis, J.J., Lehman, A.R., van der Eb, A.J. and Rommelaere, J. (1988). Les cellules de patients atteints d'ataxia telangiectasia manifestent une capacité normale de réactivation radioinduite du virus HSV-1 endommagé.
Comptes rendus de la Soc. Biologie, 181, 432-438.

- Cornelis, J.J., Becquart, P., Duponchel, N., Salomé, N., Avalosse, B.L., Namba, M. and Rommelaere, J. (1988). Transformation of human fibroblasts by ionizing radiation, a chemical carcinogen or simian virus 40 correlates with an increase in their susceptibility to the autonomous parvoviruses H-1 and Minute Virus of Mice.
J. Virol., 62, 1679-1686.

- Hilgers, G., Cornelis, J.J., Abrahams, P., Schouten, R., van der Eb, A. and Rommelaere, J. (1988). Differential reactivation and mutagenesis of single- and double-stranded DNA viruses in irradiated cells from ataxia telangiectasia patients. In : "Light in Biology and Medicine", vol. 1, Eds. R.H. Douglas, J. Moan and F. Dall' Acqua. Plenum Publ., pp. 233-240.

- Cornelis, J.J. and Rommelaere, J. (1989) Parvoviral probes for cellular responses to DNA damage. In : "Parvovirus Handbook", Ed. P. Tijssen, CRC Press, Boca Raton, in press.

- Hilgers, G., Abrahams, P.J., Chen, Y.Q., Schouten, R., Cornelis, J.J., van der Eb, A.J. and Rommelaere, J. (1989). Impaired recovery and mutagenic SOS-like responses in ataxia telangiectasia cells. Submitted to Mutagenesis

b. Indirectly related to the programme

- Mousset, S. and Rommelaere, J. (1988) Susceptibility to parvovirus Minute-Virus-of-Mice as a function of the degree of host cell transformation : little effect of Simian Virus 40 infection and phorbolester treatment. Virus Research, 9, 107-117.

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RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-103-I

Istituto Superiore di Sanità
Viale Regina Elena, 299
I - 00161 Roma

Head(s) of research team(s) [name(s) and address(es)]:

Prof. G.B. Rossi
Department of Virology
Istituto Superiore di Sanità
Viale Regina Elena, 299
I - 00161 Roma

Telephone number: 06-4990

Title of the research contract:

Radiation carcinogenesis in animals: search for and role(s) of oncogenes.

List of projects:

1. Radiation carcinogenesis in animals: search for and role(s) of oncogenes.

Title of the project no.: Radiation carcinogenesis in animals: search for and role(s) of oncogenes

Head(s) of project: Prof. Giovanni B. Rossi.

Scientific staff: Filippo Belardelli, Simonetta Pulciani, Arturo Sala, Carlo Petrini, Laura Santodonato.

I. Objectives of the project:

Many studies have been reported on the correlations between neoplastic transformation and oncogene expression in X-rav-induced tumors in experimental model systems. However, few reports have been published so far on the radiation effects on oncogene expression during tumor progression (i.e. on the possible induction of altered malignant cells within the tumor, exhibiting increased metastatic and oncogene expression) (1). Spontaneous as well as X-rav treated murine tumors have been chosen as working models to investigate the role of oncogenes in tumor onset, progression and in the metastasic process.

II. Objectives for the reporting period:

During 1988 our efforts have been devoted to the development of the following two tumor systems.

1) SJL/J model. Recentlv, we have molecularlv cloned the transforming virus, detected by transfection experiments with DNA from trasplantable cell lines from reticulum cell sarcomas (RCS) of SJL/J mice (2).

2) Friend cells model. We characterized the biologic al and biochemical differences between non-metastatic Friend leukemia cells (FLC) and highly metastatic, in vivo passaged, FLC variants (3). Our purpose was to investigate possible differences in the oncogene expression in different FLC types and to evaluate the possible use of this model to study the role of oncogenes in radiation induced tumor progression.

III. Progress achieved:

Methodology.
As described in Pulciani et al. (2), Garte et al. (4), Belardelli et al. (3).

Results.
1) SJL/J model. DNA from selected clones of NIH-3T3 transformed by the RCS virus were digested with several restriction enzymes and analyzed on agarose gels along with normal NIH-3T3 DNA. In the DNA extracted from a NIH-3T3 transformed clone (V2-6-1) a specific band of 5.6 kb was found to be present in a high copy number, upon digestion with the restriction enzyme Eco RI. We postulated that these genetic sequences represented the genome of RCS virus detected by the biological assay. Therefore, we molecularly cloned the 5.6 kb Eco RI fragment, into lambda-ZAP vector (5). Preliminary characterization of the cloned DNA suggests a high homology with mouse Polyoma viruses DNA.
2) Friend cell model and metastasis. Along with the cloning of the transforming sequences detected in RCS transplantable tumors, we looked for experimental model systems suitable to uncover the mechanisms involved in tumor progression and metastatic phenotype. Our Laboratory has deeply investigated the biologic and biochemical differences between non-metastatic, and highly metastatic, FLC variants (3). We have widely characterized a number of membrane glycoprotein changes between FLC variants exhibiting different levels of metastatic potential to the liver, or to the spleen, of syngeneic mice (3). Briefly, the following major FLC types have been isolated and extensively characterized: 1) original non metastatic FLC; 2) in vivo passaged FLC, highly metastatic to the spleen and to the liver; 3) non metastatic WGA-resistant FLC (derived from the in vivo passaged FLC). All these tumor cell variants form solid tumors when injected s.c. in DBA/2 mice, but they exibit a completely different pattern of invasion. In the course of 1988 we have further characterized the membrane glycoprotein changes of metastatic and non metastatic FLC. In particular we have found a strong correlation between the sialylation of a 150 kD glycoprotein and the acquisition of the metastatic phenotype of FLC to the liver (6). Moreover, an increased expression of H-2 (class I) antigens was found to be correlated with the highly metastatic phenotype of the in vivo passaged FLC (6). Recent results obtained in our Laboratory indicate that an increased expression of the c-myc oncogene is associated with the highly metastatic phenotype of FLC (6). Possible changes in the expression of other oncogenes are under investigation using this tumor model system.

Discussion.
1) SJL/J model. The data obtained from the DNA sequencing of the virus isolated from RCS transpantable cell lines suggest that it is a member of Papova virus family therefore a probable biological contaminant of transplantable tumors tested in the transfection experiments (2). SJL/J mice have been irradiated with 1.5 Gy four times weekly, and checked to determine tumor development. In particular we wanted to investigate the influence of X-rays on the expression of the endogenous retroviral sequences. We shall not pursue this objective, since the molecularly cloned virus appeared not related to the onset of spontaneous RCS tumors.

2) Friend cell model and radiation effects on metastasis. The data obtained from the biochemical comparison of the non-metastatic and highly metastatic FLC clones suggest that proto-oncogene expression may be involved in tumor progression to the metastatic phenotype as well as tumor onset. Therefore we would like to further investigate the described FLC variants. In particular we would like to explore the possible effects of sublethal doses of X-rays on the in vivo behaviour and oncogene expression in different FLC types. The treated FLC clones will be tested for oncogene expression, tumorigenic potential and membrane glycoprotein pattern. It is reasonable to assume that the proposed studies in the FLC model system will provide some insights on the possible effects of X-ray irradiation on tumor progression events and oncogene expression.

References.

1. Nowell P.C. (1986). Mechanisms of tumor progression. Cancer Res. 46, 2203-2207.

2. Pulciani S., Sakamo T., Ohnishi K., Anastasi A.M., Pecorelli A., Fiorucci G., Oppi C., Rossi G.B. and Bonavida B. (1987). Detection of a transforming gene in spontaneous reticulum cell sarcoma of SJL/J mice: genetically linked and host-dependent neoplasia. Cancer Res. 47, 573-576.

3. Belardelli F., Ferrantini M., Maury C., Santurbano L., Gresser I. (1984). On the biologic and biochemical differences between in vitro and in vivo passaged Friend Erythroleukemia Cells. Tumorigenicity and capacity to metastasize. Int. J. Cancer 34: 389-395.

4. Garte S.J., Savey M.J. and Burns F.J. Oncogenes activated in Radiation-Induced (1987) Rat Skin Tumors in "Radiation Carcinogenesis and DNA Alterations" Edited by Burns F.J., A.C. Upton and G. Silim. Nato ASI Series Vol. 124 Plenum pus. pp. 389-397.

5. Maniatis T., Fritsch E.F., Sambrook J. (1982). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, New York.

6. Ferrantini M., Pulciani S., Proietti F., Lespinats G., Anastasi A., Ciolli V., Rizza P. and Belardelli F. (1989). Studies on the expression of H-2 antigens in non metastatic and highly metastatic Friend erythroleukemia cells. Correlation with the in vivo behaviour of the tumor cells. Clin. and Exp. Metastasis, in press.

IV. Objectives for the next reporting period:

Non metastatic FLC will be treated with low doses of x-rays and/or low dose rates. Viable irradiated cells will be either directly injected i.v. in mice or cloned in vitro, before injection. The in vivo selection of radiation-induced malignant tumor cells will be performed by i.v. injection of 10^6 FLC (such tumor cell inoculation does not generally result in any spleen or liver metastasis using non-irradiated tumor cell counterparts). Likewise, highly metastatic FLC will be irradiated, in the same experimental conditions. Possible changes in the expression of the c-myc oncogene, and other oncogenes, such as ras and myb, will be analyzed and correlated with the in vivo behaviour of tumor cells.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Benjamin Bonavida Dr. Jonathan Kartz	UCLA, Department of Microbiology and Immunology UCLA School of Medicine, Center for the Helth Sciences, Los Angeles, California 90024.
Dr. Orazio Sapora	Laboratorio di Tossicologia comparata e Ecotossicologia, Reparto di Tossicità cellulare d'organo e di organismo. Istituto Superiore di Sanità, Roma.

VI. Publications:

1. Benedetto A., Elia G., Sala A. and Belardelli F.
Hyposialylation of high molecular weigh membrane glycoproteins parallels the loss of metastatic potential in wheat germ agglutinin-resistant Friend leukemia cells.
Int. J. Cancer, 1989, in press.

2. Ferrantini M., Pulciani S., Proietti E., Lespinats G., Anastasi A., Ciolli V., Pizza P. and Belardelli F.
Studies on the expression of H-2 antigens in non metastatic and highly metastatic Friend erythroleukemia cells. Correlation with the in vivo behaviour of the tumor cells.
Clin. and Exp. Metastasis, 1989, in press.

3. Ciotta C., Pizza P., Ferrantini M., Puddu P., Proietti F. and Belardelli F.
Aumentata espressione del c-myc e dei geni H-2 di classe I in cellule eritroleucemiche di Friend con alta capacita' metastatizzante selezionate mediante passaggi in vivo.
VII Congresso Nazionale della Associazione di Biologia Cellulare e del Differenziamento. Spoleto, 16-19 Ottobre 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-089-UK

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB - Oxon OX11 ORQ

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Stather
Biomedical Effects Department
NRPB
Chilton, Didcot
GB - Oxon OX11 ORQ

Telephone number: 0235-831600

Title of the research contract:

The dosimetry and metabolism of incorporated radionuclides.

List of projects:

1. The dosimetry of inhaled radionuclides.
2. Metabolism and dosimetry of radionuclides in bone.
3. Mechanical transport of particles from the respiratory tract.
4. Translocation of material from particles deposited in the respiratory tract.

Title of the project no.: 1

The dosimetry of inhaled radionuclides

Head(s) of project:

Dr. G.N. Stradling

Scientific staff:

Miss S.A. Gray
Mr. A. Hodgson
Mr. J.C. Moody
Ms. M. Ellender
Ms. C.G. Collier

I. Objectives of the project:

To examine, in rodents, the behaviour of actinides that could be inhaled by humans as a result of occupational exposure or after their release into the environment. To undertake studies designed to understand the mechanisms involved in the translocation and clearance of radionuclides from the various regions of the respiratory system. To provide an experimental basis for assessing intakes of plutonium and the higher actinides.

II. Objectives for the reporting period:

1. To study the lung clearance of plutonium (Pu), americium (Am) and associated radionuclides in rodents after the alveolar deposition of residues produced or likely to be produced at nuclear facilities.

2. To investigate the efficacy of a pure form of LICAM-C on the decorporation of Pu and Am inhaled as their nitrates.

III. Progress achieved:

Methodology

The facility for administering aerosols to small animals described in the previous report has been used extensively during the year for our continuing programme on the metabolism of industrial actinide bearing dusts. The aim of this work is to provide an experimental basis for recommending site specific annual limits on intake and to facilitate the interpretation of chest monitoring or bioassay data. To predict the behaviour of the actinides in humans, their rates of transfer to blood are combined with the rates of mechanical clearance from the lungs obtained from human volunteers who have inhaled ^{85}Sr and ^{86}Y labelled fused clay particles. A similar methodology is likely to be proposed by an ICRP Task Group. In addition the relationship between the systemic content of the actinides and cumulative urinary excretion is evaluated to assess whether current models for interpreting urine data are appropriate. The facility has been, and will be, used to investigate the effect of promising new chelating agents on the decorporation of Pu and Am inhaled in different chemical forms. Where additional information is required on the relationship between the systemic content of the actinides and their cumulative excretion, or when insufficient material is available for inhalation experiments, the material will be administered by intratracheal instillation.

Results and discussion

After the inhalation of $^{239}PuO_2/^{241}AmO_2$, lung retention data for rats are complete up to 72 weeks and for guinea pigs up to 60 weeks. In the rat experiment, the lung content of ^{239}Pu has decreased to 3.4% of the initial deposit whilst the ^{239}Pu to ^{241}Am ratio in the material retained in the lungs has only decreased by about 7% indicating the closely similar biokinetics of these radionuclides. In the guinea pig experiment the ^{239}Pu to ^{241}Am ratio in the lungs has decreased by about 2%.

After the inhalation of Nevada test site material, lung and tissue retention data for rats killed up to 365d after inhalation are complete. At this time only about 5% of the initial deposit of ^{239}Pu has been retained by the lungs; the increase in the ^{239}Pu to ^{241}Am ratio is about 48%. About 16% and 20% respectively of the ^{239}Pu and ^{241}Am are estimated to have translocated to the blood reflecting the moderate transportability of the radionuclides. The rate of translocation of ^{239}Pu to blood between 28 and 365d was estimated to be 0.13% d^{-1} (Table 1). The results indicate that the material more closely resembles a class W than a class Y compound. The increase in the ^{239}Pu to ^{241}Am ratio in the lungs of guinea pigs at 1 year is about 17%. After intratracheal injection of a suspension of the material into the lungs of rats, the relationship between the systemic content of ^{239}Pu and cumulative urinary excretion at 7d and 21d is similar to that obtained after the intravenous injection of ^{239}Pu citrate.

The metabolic behaviour of residues obtained from a cooling pond and decanning facility at a nuclear facility have been studied in rats. The committed doses after inhalation by workers will result mainly from the actinides even though some fission products such as ^{144}Ce and ^{137}Cs are present in considerable excess. The ratio of ^{239}Pu to ^{241}Am and ^{144}Ce retained in the lungs of rats after 1 year increased by less than a factor of 2. The rates of translocation of ^{239}Pu, ^{241}Am and ^{144}Ce lie between those predicted for class W and Y compounds (Table 1). The behaviour of ^{137}Cs was similar to that of a class D compound.

The predicted behaviour of these radionuclides in humans suggest that the measurement of ^{144}Ce in the chest and ^{137}Cs in the body could be used to assess intakes of the actinides at well below the annal limit (ALI).

Table 1. Transfer rates of ^{239}Pu from the lungs of rats to blood and the values for class W and Y compounds derived from the ICRP model for man.

Material	Rates of transfer (% d^{-1}) 0 - 28d	28-365d
$^{239}PuO_2$	0.007	0.007
Nevada test site	0.31	0.13
Pond water residues	0.21	0.12
Decanning residues	0.17	0.10
Class W compound	1.5	0.45
Class Y compound	0.067	0.016

A study on the efficacy of a pure form of LICAM(C) for enhancing the elimination of ^{238}Pu and ^{241}Am from the rat after their inhalation as the nitrate showed that it was substantially inferior to DTPA. Using the same treatment regimen (30 μmol kg^{-1} administered ip at 0.02, 0.25, 1, 2 and 3d and then twice weekly from 6d to 24d) the total body content of ^{238}Pu and ^{241}Am at 28d were about 2% of those in untreated animals using DTPA, and about 50% and 93% respectively using LICAM(C). The efficacy of pure LICAM(C) was only marginally better than when using the esterified or polymeric forms reported previously. The results obtained for DTPA represent the best therapeutic effect ever achieved in animal experiments.

The administration of disodium 4,5-dihydroxy - 1,3 benzene-disulphonic acid (Tiron) has been suggested as an antidote for soluble forms of uranium. We have investigated, in rats, the efficacy of this substance after the administration of uranium in amounts which correspond to about 20 times the permissible daily intake but have found that the body content is only reduced by about 60% when compared with untreated animals.

IV. Objectives for the next reporting period:

Continuation of studies on the metabolic characteristics of Pu, Am and U present in residues formed during nuclear fuel production or reprocessing.

Biokinetics of uranium deposited as U_3O_8 and UO_2 in the lungs of the baboon; in collaboration with the CEA.

Bioavailability of plutonium and americium at Palomares in collaboration with the Ministry of Industry and Energy, Madrid.

Biokinetics of thorium. The industrial use of monazite and other thorium bearing materials is one of increasing concern. Initially it is intended to review the extent of this problem.

Investigation of the efficacy of the chelating agent 3,4,3-LIHOPO on the decorporation of Pu and Am; this substance has been synthesised at the University of California where screening experiments with animals have shown it to be far superior to DTPA after its injection or oral administration.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. H. Métivier, Laboratoire de Toxicologie Experimentale, CEA Bruyeres-le-Chatel, France.

Prof. D.M. Taylor and Prof. V. Volf, Institut fur Genetik und Toxikologie, KFK Karlsruhe, Federal Republic of Germany.

Prof. O. Vanderborght, Dept. of Radiobiologie, Belgian Nuclear Center, Mol, Belgium.

Prof. K. Raymond, College of Chemistry, University of California, Berkeley, CA.

Dr. P. Durbin, Lawrence Livermore Laboratory, Berkeley, CA.

VI. Publications:

The metabolism of ceramic and non-ceramic forms of uranium dioxide after deposition in the rat lung. G.N. Stradling, J.W. Stather, S.A. Gray, J.C. Moody, A. Hodgson, D. Sedgwick and N. Cooke. Human Toxicol. 7, 133-139.

The use of animal experiments for assessing annual limits on intake and interpreting chest monitoring data for workers exposed to industrial actinide bearing dusts. G.N. Stradling and J.W. Stather. Proc. 26th Hanford Life Sciences Symposium. Health Physics, in press.

Limits on intake and the interpretation of monitoring data for workers exposed to industrial uranium bearing dusts. G.N. Stradling, J.W. Stather, A. Price and N. Cooke. Radiat. Prot. Dosim. in press.

Assessment of intake of an actinide bearing dust formed from the pond storage of spent magnox fuel. G.N. Stradling, J.W. Stather, S.A. Gray, J.C. Moody, M. Ellender and C.G. Collier. ibid.

The efficacies of pure LICAM(C) and DTPA on the retention of plutonium-238 and americium-241 in rats after their inhalation as nitrate and intravenous injection as citrate. G.N. Stradling, J.W. Stather, V. Volf and D.M. Taylor. To be submitted to Int. J. Radiat. Biol.

The biological assessment of occupational exposure to actinides. Report on an International Workshop held at Versailles from May 30th - June 2nd 1988. G.N. Stradling and A. Birchall. Int. J. Radiat. Biol. 54, 1053-1057, 1988.

Title of the project no.: 2

Metabolism and Dosimetry of Radionuclides in Bone

Head(s) of project:

Dr. R.D. Saunders

Scientific staff:

Ms. M. Ellender
Miss J.W. Haines

I. Objectives of the project:

To improve our understanding of the behaviour of bone-seeking radionuclides in the skeleton.

To provide information that can be used to improve assessments of doses to radiation sensitive cells in the skeleton following intakes of bone-seeking radionuclides.

II. Objectives for the reporting period:

To continue studies on the distribution of ^{239}Pu, ^{241}Am and ^{233}U in CBA/H mice.

To begin studies of the induction of ostesarcomas in CBA/H mice by alpha emitting, bone-seeking radionuclides.

III. Progress achieved:

Methodology

The tissue distribution and retention characteristics of ^{239}Pu, ^{241}Am and ^{233}U up to 448 days after intraperitoneal administration to male and female mice have been determined.

Detailed autoradiographic studies of the distribution of these radionuclides with time in two bones, the femur and lumbar vertebrae, are in progress. Difficulties have arisen in the preparation of conventional autoradiographs of these bones. Further mice have been injected with the three radionuclides for conventional autoradiography of the smaller mandibular condyle and rib which have been found more suitable for this technique.

The main effects study to examine the comparative toxicity of plutonium-239, americium-241 and uranium-233 in mice using the induction of osteosarcoma as a toxicological end-point is now underway. Groups of 50-100 mice have been given serial intraperitoneal injections of the three radionuclides over a three week period; a control group have been similarly injected with saline. Total administered activity levels of plutonium citrate of 25, 15 and 5 kBq kg^{-1} have been given. These levels of plutonium are expected to produce an osteosarcoma incidence of 40, 15 and 7.5% respectively. In order to give the same average skeletal dose to plutonium, americium citrate has been given at total activity levels of 28.9, 17.2 and 5.8 kBq kg^{-1} and uranium citrate at total activity levels of 197.3, 117.6 and 39.5 kBq kg^{-1}. The average skeletal bone doses at 600 days are expected to be 0.63 Gy, 0.37 Gy and 0.125 Gy for the high medium and low groups respectively. The mice are being followed until they become moribund or develop a visible tumour or paralysis when they are killed. At death, all animals have a post-mortem examination and will be X-rayed to check for bone-tumours.

A further group of animals has been injected with 25 kBq kg^{-1} plutonium-239 and will be X-rayed periodically.

Results and discussion

Peak skeletal activity occurred 28 days after injection with plutonium and americium and 7 days after injection with uranium. After 448 days, the total retention of administered activity in male mice was 19.6% for plutonium, 20.7% for americium and 2.5% for uranium. The proportion of this activity retained by the skeleton was 86.1% for plutonium, 94.3% for americium and 99.4% for uranium. Female mice were found to have similar retention characteristics.

Autoradiographic studies of the distribution of these radionuclides with time in the femur and lumbar vertebrae have been carried out. CR39 autoradiography has shown that plutonium is deposited predominantly on the endosteal bone surfaces. During remodelling and redistribution some burial of the plutonium was seen at the epiphyseal plates and in cortical bone, but activity appears to remain largely on endosteal bone surfaces. With time, increasing amounts accumulate in the macrophages in the bone marrow. Americium was seen to deposit more evenly on endosteal and periosteal bone surfaces and also on the internal bone surfaces of vascular canals in the cortical bone. With time, some burial was seen at the growth plate and small amounts of activity accumulated in the bone-marrow macrophages. Uranium appeared to deposit on both periosteal and endosteal bone surfaces

but the distribution was uneven with concentrations of activity in some areas. Small amounts of diffuse activity are seen throughout the bone marrow and mineral.

A small number of animals in the toxicity study have died or been killed. Most of these deaths have been due to liver hepatomas which are endemic in CBA/H mice.

IV. Objectives for the next reporting period:

Studies on the distribution of plutonium-239, americium-241 and uranium-233 in the CBA/H mouse skeleton will continue.

The main toxicity study will continue. The animals will be followed until they develop tumours, paralysis or become moribund when they will be killed. All animals have a post-mortem examination and will be X-rayed to check for bone-tumours.

The group of mice injected with 25 kBq kg^{-1} of plutonium will be examined by X-radiography once a month to localise the sites of tumour induction and follow the tumour growth rate.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. E. Humphreys and Miss V. Stones
MRC Radiobiology Unit, Harwell, Didcot, Oxon, UK

VI. Publications:

Ellender, M. and Haines, J.W. The retention and distribution of ^{233}U, ^{239}Pu and ^{241}Am in the CBA mouse. Abstract of a presentation given to a meeting of the EULEP Task Groups 8, 9 and 16 held at Chilton, 7th-8th November 1988. To be published in the EULEP Newsletter.

Title of the project no.: 3

Mechanical transport of particles from the respiratory tract

Head(s) of project:

Dr. M.R. Bailey

Scientific staff:

Dr. R.A. Bulman, Miss C.G. Collier, Mr. N. Dodd, Miss M. Dorrian, Dr. G. Etherington, Ms. S. Gray, Mr. N. Green, Mr. A. Hodgson, Dr. J.R.H. Smith

I. Objectives of the project:

The overall objective of the project is to improve the scientific basis of models used to relate intakes of radionuclides by inhalation to tissue doses and to environmental and bioassay measurements, by:

(i) Measuring the rate at which discrete particles are cleared from different regions of the human respiratory tract: nasal passage, bronchial tree and pulmonary region.

(ii) Testing the hypothesis that the rates at which discrete particles are cleared from the lungs to the gastro-intestinal tract are independent of particle composition.

(iii) Investigating mechanisms of particle clearance from the lungs, and factors affecting clearance, including inter-species differences.

II. Objectives for the reporting period:

Continuation of the study of the biokinetics of intravenously administered ^{88}Y.

Further development of techniques required to conduct the approved studies of particle clearance from the human nasal passage, and commencement of the experiments themselves.

Further investigation in rodents of factors affecting mechanical transport rates.

Compilation and analysis of measurements of the lobar distribution of particles in the lungs of rodents as a function of time after administration.

III. Progress achieved:

Methodology

Progress has been made on developing the technique needed to conduct the human volunteer study of the deposition and clearance of inhaled particles in the nasal passage, for which approval was given during the last reporting period. Subjects will inhale FAP or polystyrene particles of uniform size labelled with ^{97}Ru ($t_{½}$ 2.8 d). Retention in the nasal passage will then be followed by external counting of gamma-rays for at least 24 hours.

The facilities for producing monodisperse, radioactively-labelled particles, and for administering them to human volunteers under controlled conditions, are being extensively upgraded. Equipment is being set up for separating ^{97}Ru from cyclotron-irradiated molybdenum powder by steam distillation. HPLC procedures were developed for purifying a ruthenium-binding lipophilic chelate identified for labelling polystyrene with ^{97}Ru. A Vibrating Orifice Aerosol Generator (VOAG) was obtained for producing monodisperse aerosols, as it is capable of producing larger particles than the spinning-disc generator used hitherto. Initial trials confirmed that the VOAG can also achieve a higher degree of uniformity, and a higher output efficiency than the spinning-disc. The Aerodynamic Particle Sizer was upgraded, raising the upper limit of its size range to 30 μm, and reducing its response time to ~1 s, enabling the concentration and size distribution of the aerosol to be monitored during administration to subjects. A laser aerosol photometer/spirometer, designed specifically for measurements of particle deposition in the respiratory tract, was obtained from Prof. Stahlhofen's group (GSF, Frankfurt). This instrument, with other components of the aerosol administration apparatus will be operated under computer control. Software was written to enable rapid, simultaneous measurements to be made of the flow rate and aerosol concentration in inhaled and exhaled air. Methods for calibrating the photometer/spirometer are being developed.

A detector system is being set up to enable measurements of activity in the head, lungs and stomach to be made during and immediately after administration of labelled aerosol. Detector support stands designed for this purpose were made and five 120 x 100 mm (NaI(Tl) detectors installed. In each experiment a rapid series of simultaneous measurements has to be made with several detectors and the accumulated spectra stored. A personal computer based multi-channel analyser (MCA) was obtained, as in this application it offers major advantages over a conventional MCA.

Although these facilities are currently required for the study of deposition and clearance of inhaled particles in the human nasal passage, they will be applicable to projected future human inhalation studies, especially those requiring particle administration to a selected part of the respiratory tract.

Results and Discussion

(i) Measurement of particle clearance rates from different regions of the human respiratory tract.

The study of the biokinetics of ^{88}Y has continued. ^{88}Y is employed as a label for the fused aluminosilicate particles (FAP) used in long-term lung clearance studies. To determine the contribution to clearance made by particle dissolution from measurements of urinary excretion of the label

requires information on the retention and excretion of ^{88}Y following systemic uptake. In this study, subjects were intravenously injected with 0.5 ml of 0.9% sodium chloride solution to which ^{88}Y citrate at ph 7 was added. Measurements on two subjects, the first receiving 0.4 kBq in a pilot experiment and the second receiving 4.0 kBq, continued for 6 months and one year respectively, and have now been completed. The results of the pilot experiment were consistent with the more accurate results obtained for the second subject. Another subject has been injected with 4.0 kBq of ^{88}Y, and measurements on him are nearing completion. As before, total body and organ (liver, bone and bladder) retention of ^{88}Y are measured using NaI(Tl) detectors in a low-background enclosure. Quantitative faecal and urinary collections were made for 14 and 24 days after intake respectively, and 72 hour urine samples are now being collected at monthly intervals. Very good agreement was found between the results for the second and third subjects. The retention function for total body activity in both cases is well characterised by a two-component exponential of the form:

$$R(t) = 0.20 \exp(-\ln(2)t/T_1) + 0.80 \exp(-\ln(2)t/T_2)$$

where T_1 = 18 hours and T_2 ~ 3 years.

Excretion measurements are in good agreement with this retention function. The fraction of ^{88}Y excreted during the first 5 days which appeared in urine was 0.94 and 0.93 for the two subjects. Initial uptake to liver was also very similar at 12% and 10% of the injected activity. For both subjects, approximately half of this activity was retained in the liver with a long half-time compared to the time scale of the experiment. The main difference observed between the two subjects is in the half-time of the short term component of retention in the liver (600 hours and 150 hours). Measurements of the distribution of activity in the body at various times during the experiment are consistent with most of the remaining activity being deposited on bone surfaces.

For comparison, 4 rats were intravenously injected with the same ^{88}Y solutions. Urine and faeces were collected for 4 days, and the animals killed to determine the tissue distribution. Generally results were similar to those in the human subjects: about 25% of the injected activity was excreted in urine, mainly on the first day, and 8% in faeces. About 7% of the activity retained in the body was in the liver.

(ii) Animal experiments

Information on factors affecting mechanical transport rates were provided by the lung clearance studies described under Project 4. The rate of transport of particles from the lungs to the GI tract was measured following inhalation of ^{57}Co-labelled Co_3O_4 by rats aged 3, 13, 21 and 46 weeks. In all groups the clearance rate decreased from ~3% of the remaining lung content per day at a week after inhalation, to ~0.5% per day at 9 months. Analysis of variance showed no significant differences in the clearance rate between the age groups. The mechanical clearance rates observed here were similar to those observed in rats in the previous study using a more porous form of cobalt oxide, and in studies using FAP, which dissolve much more slowly in vivo than cobalt oxide particles. The results thus indicate that in a given species mechanical transport rates are independent of age, and provide further support for the assumption that they are independent of particle composition.

IV. Objectives for the next reporting period:

Completion of the study of the biokinetics of intravenously administered ^{90}Y.

Further development of techniques required to conduct the approved studies of particle clearance from the human nasal passage, and commencement of the experiments themselves.

Compilation and analysis of measurements of the lobar distribution of particles in the lungs of rodents as a function of time after administration.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. W. Stahlhofen,
Abteilung für Biophysikalische Strahlenforschung, GSF Frankfurt, West Germany.

VI. Publications:

The effect of alpha irradiation on the retention of particles in the rat lung. C.G. Collier, A.C. James and M. Ellender. Ann. occ. Hyg. 32, Supplement 1 (Inhaled Particles VI) 1007-1015, 1988.

Factors affecting the clearance of fused aluminosilicate particles from the rat lung. C.G. Collier, A. Hodgson, M.R. Bailey and S.F. Barry. J. Aerosol Sci. 19, 689-702, 1988.

Title of the project no.: 4

Translocation of material from particles deposited in the respiratory tract

Head(s) of project:

Dr. M.R. Bailey

Scientific staff:

Miss C.G. Collier
Mr. A.M. Ball
Ms. S. Gray

I. Objectives of the project:

The overall objective of the project is to improve the scientific basis of models used to relate intakes of radionuclides by inhalation to tissue doses and to environmental and bioassay measurements, by:

(i) Comparing in different species the rate of translocation of material from particles in the lung to the blood.

(ii) Measuring absorption into the blood of materials deposited in the anterior or posterior nasal passage of human subjects.

(iii) Measuring absorption into the blood and retention in the epithelium of materials deposited in the nasal passages of rodents.

II. Objectives for the reporting period:

To complete a study of the effect of age on lung clearance of cobalt oxide. To investigate factors affecting the dissolution of cobalt oxide in the lung and the translocation of cobalt from the lungs to the blood, and develop a biokinetic model for the behaviour of cobalt following inhalation of the oxide. To select and prepare a material for another interspecies comparison and carry out preliminary tests.

To commence experiments to measure absorption into the blood and retention in the nasal epithelium of materials deposited in the nasal passage of rodents.

III. Progress achieved:

Methodology

Assessments of exposure of the general public by radionuclides released to the environment require age-specific information on factors determining the intake of radionuclides and their subsequent behaviour in the body. The experimental study of the effect of age on alveolar clearance of inhaled particles initiated during the last reporting period has been completed. Monodisperse ^{57}Co-labelled cobalt oxide particles with a mean geometric diameter of 1.0 μm were provided for use in this experiment by Dr. W.G. Kreyling (GSF, Neuherberg). Four groups of rats (N = 11-16) aged 3, 13, 21 and 46 weeks, were simultaneously exposed to an aerosol of the particles by a single brief nose-only inhalation.

Results and Discussion

Whole body activity, and faecal and urinary excretion rates were determined at frequent intervals up to 9 months after exposure. To determine the distribution of ^{57}Co in the body, two or more animals from each group were killed at 7, 83, 182 and 281 days after exposure. The fraction of the whole body activity present in the lungs at death F was determined by whole body counting the animals before and after removal of the lungs. In all sacrificed groups, except for the youngest rats at 281 days, F was greater than 90%. For each animal, lung retention as a fraction of that at 7 days after inhalation was determined from its measured whole body activity and interpolated values of F. Mean values for each group at selected times are given in the Table. Two way analysis of variance showed that the youngest animals had significantly lower retention throughout the experiment than the other groups.

Table of results

Age exposed (weeks)	Lung retention, % lung retention at 7 days (mean ± SD)			
	1 month	3 months	6 months	9 months
3	57.1 ± 3.3	16.5 ± 1.2	3.31 ± 0.43	1.36 ± 0.27
13	58.0 ± 2.4	20.7 ± 2.4	5.24 ± 0.84	1.94 ± 0.34
21	56.0 ± 2.5	21.0 ± 2.0	5.30 ± 0.89	
46	62.8 ± 3.0	25.7 ± 2.5	6.57 ± 0.80	2.50 ± 0.33

Supplementary experiments were conducted on animals of ages similar to those in the inhalation study, to determine the retention and excretion of ^{57}Co following systemic uptake, and the uptake of ^{57}Co from particles passing through the GI tract. Transfer coefficients were derived for the fractions of ^{57}Co excreted in urine and faeces in each case. Regression analysis on the results showed some small but significant differences between age groups, but as no linear trend with age was found, the results from all groups were combined to give best estimates of the values.

These transfer coefficients were used to estimate ^{57}Co lung clearance rates from the measured urinary and faecal excretion rates. The rate of translocation of ^{57}Co from the lungs to the blood (S(t) at time t after inhalation) and the rate of mechanical transport of particles from the

lungs to the GI tract, were calculated as fractions of the activity remaining in the lungs. In all groups S(t) increased throughout the experiment, from ~1% d^{-1} initially to 1.5-4% d^{-1}. The youngest animals consistently showed the highest values of S(t), up to 4 times the values in the other age groups. Analysis of variance showed significant differences between the age groups, with the greatest contribution coming from the youngest animals. As reported under Project 3, no effect of age on mechanical clearance rates was observed.

These results indicate that there is little effect of age on lung clearance kinetics in mature animals, but that the rate of translocation of material from lung to blood may well be higher in young animals. Consideration should be given to this possibility in evaluating doses to children resulting from exposure to airborne radioactive material.

This study also forms part of a second interspecies comparison of lung clearance co-ordinated through the European Late Effects Project Group (EULEP). The batch of Co_3O_4 used has also been employed in inhalation studies in beagle dogs at G.S.F. Neuherberg, and in baboons at C.E.A. Bruyêres-le-Châtel. Lung retention and clearance rates measured here in adult rats are being compared with the results in dogs and baboons. The results are also being compared with those obtained in the previous intercomparison, in which the Co_3O_4 particles were more porous, and hence of lower density.

Important differences between species in the rate of translocation from lung to blood were observed in these studies, and therefore factors affecting the dissolution of particles in the lung and the translocation of dissolved material to the blood are being investigated. Since the dissolution of particles in the lung takes place mainly within the phagolysosomes of alveolar macrophages (AM), the pH of the phagolysosomal solution may well influence the dissolution rate either directly, or in conjunction with other components such as complexing agents. Techniques for obtaining AM by lavage and for maintaining them are being developed. On average 4, 9 and 50 million AM were recovered from the young rat, adult rat and guinea pig respectively. They could be maintained for up to 24 h with 85% viability either free in RPMI culture medium, or adhered to glass cover slips. An interspecies comparison of the intraphagolysosomal pH of AM was organised through EULEP. AM from rat and guinea pig obtained at this laboratory, and from baboon, dog and rabbit obtained at the other collaborating laboratories were taken to the Karolinska Institute, Stockholm on the same day. The cells were incubated with fluorescein-labelled silica particles, and the pH within the phagolysosomal vacuole was determined by UV fluorescence spectrophotometry. Results were obtained in most species and are being analysed and collated, but preliminary data suggest that there is little difference between species.

The collaborative study to compare lung clearance in different species of monodisperse, ^{57}Co-labelled cobalt oxide particles with geometric diameters of 0.8 and 1.7 μm has been completed. Further effort was put into co-ordinating and summarising the results from the various collaborating laboratories to produce a final report for publication.

IV. Objectives for the next reporting period:

To investigate factors affecting the dissolution of cobalt oxide in the lung and the translocation of cobalt from the lungs to the blood, and develop a biokinetic model for the behaviour of cobalt following inhalation of the oxide.

V. Other research group(s) collaborating actively on this project (name(s) and address(es)):

Dr. H. Métivier, Dr. D. Nolibe,
CEA/IPSN/SPE/STCE Bruyêres-le-Châtel, France.

Dr. W.G. Kreyling,
Institut für Strahlenschutz, GSF Neuherberg, West Germany.

Dr. P. Camner, Dr. M. Lungborg, Dr. K. Nilsen,
Karolinska Institute, Stockholm, Sweden.

VI. Publications:

An interspecies comparison of the translocation of material from lung to blood. M.R. Bailey, W.G. Kreyling, S. Andre, A. Batchelor, A. Black, C.G. Collier, E. Drosselmeyer, G.A. Ferron, P. Foster, B. Haider, A. Hodgson, H. Métiver, S.R. Moores, A. Morgan, H-L. Müller, G. Patrick, S. Pickering, D. Ramsden, C. Stirling and R.J. Talbot. Ann. Occ. Hyg., 32, Supplement 1 (Inhaled Particles VI) 975-985, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-196-I

Università di Roma "La Sapienza"
Piazzale Aldo Moro 5
I - 00185 Roma

Head(s) of research team(s) [name(s) and address(es)]:

Prof. R. Strom
Dipartimento di Biopatologia Umana
Università di Roma "La Sapienza"
Viale Regina Elena 324
I - 00161 Roma

Telephone number: 06-493776

Title of the research contract:

Regulation of DNA methylation in cell differentiation, transformation and repair.

List of projects:

1. Regulation of DNA methylation in cell differentiation, transformation and repair.

Title of the project no.:

B16 - D - 196 - I

Regulation of DNA methylation in cell differentiation, transformation and repair.

Head(s) of project:

Prof. Roberto Strom
Dipartimento di Biopatologia Umana
Università di Roma "La Sapienza"
Viale Regina Elena, 324 - 00161 Roma (Italy)

Scientific staff:

Dr. E.P. Whitehead, Prof. P. Calafa, Prof. A. Fiori,
Dr. S. Mastrantonio, Prof. C. Salerno, Dr. D. Carotti,
Dr. P. Allegra, Dr. F. Palitti, Dr. S. Scarpa.

I. Objectives of the project:

Study of the regulation of DNA methylation, in an in vitro differentiating cell system, by agents which interfere with the differentiation process, and in particular by sublethal exposure of the cells to ionizing radiations.

II. Objectives for the reporting period:

1) Effects of sublethal exposure to X-irradiation on specific gene expression by cultured cells endowed with a DNA-methylation-sensitive tendency to undergo terminal differentiation.
2) Effects of DNA methylation inhibitors on the susceptibility to radiation damage of cultured mammalian cells.
3) Evaluation of single-strand breaks occurring in nuclear DNA during cell differentiation and/or exposure to X-rays.
4) Identification of factors which regulate, within chromatin structure and at the single gene level, the susceptibility to in vitro methylation of particular CpG sequences in DNA.

III. Progress achieved:

1. Methodology.

Besides the methodology described in previous reports (*In vitro* culture and characterization of various clones of the L5 myoblast cell line; induction of myoblast differentiation *in vitro*; determination of 5-methylcytosine levels in DNA; isolation and characterization of different fractions of eukaryotic chromatin; regulation of the activity of DNA-methyltransferase on various DNA sequences and in different chromatin fractions), a further characterization of the nuclear components of the differentiating and/or irradiated myoblasts was obtained by making use of a DNA alkaline elution technique: the dividing cells, pre-labelled for 48 hours with ^{14}C thymidine and exposed to different doses of x-rays, were removed from the flasks, placed on Nuclepore polycarbonate filters and lysed at pH 8.6 with sodium dodecylsulfate and proteinase K, so as to allow differential elution from the filters, using eluting buffers of increasing pH values, of distinct DNA fractions.

2. Results and Discussion

Myoblasts in fusing conditions (i.e. transferred from "growing" medium to a medium containing 1% -- rather than 10% -- foetal calf serum) showed, when irradiated with 2,5 and 5 Grays at days 1, 2, 3, or 4 after transfer, a marked but gradual decrease, from day 1 to day 4, of the x-rays inhibitory effect on cell fusion. This observation is consistent with the hypothesis that cell commitment to differentiation is inversely related to the susceptibility of the cells to agents interfering with differentiation itself. Cells which had completed commitment, even if they were not yet fused (this event becoming evident only at day 6), were insensitive to irradiation, up to 5 Grays.

Since the myoblast system is usually stimulated to differentiation and fusion by 3 deazaadenosine (DZA), an inhibitor of enzymatic methylation pathways, and since this effect was enhanced by homocysteine (HCY) -- presumably through accumulation of the deaza analog of S-adenosylhomocysteine -- the effects of DZA and/or HCY on cell sensitivity to x-rays were investigated. HCY was shown to act as a protecting agent against radiation damage; this effect could not however be ascribed to mere radiochemical protection, since its magnitude was the same whether HCY had been added before or immediately (within 20 minutes) after irradiation. Furthermore, HCY by itself actually caused a slight decrease of myoblast differentiation, while together to DZA it acted. In most myoblast clones, as a fusion stimulator. The presence of HCY and/or DZA in the culture medium of cells which were exposed to x-rays after completion of their commitment to differentiation, fully abolished the inhibitory effect of radiation on the fusion process, the final levels of multinucleated myofibers being at least equal to those of non-irradiated controls.

In parallel experiments the DNA alkaline elution technique was also used, on the same system of differenting myoblasts, both to monitor radiation-induced single-strand breaks of DNA, and to detect more complex structural differences in chromatin between undifferentiated and differentiated cells. It was sofar shown that:

1) the impulse to differentiation produces a chromatin rearrangement, indicated by the presence, in differentiated cells, of a consistent genomic fraction (approximately 30% of total chromatin) highly sensitive to alkaline denaturation -- this fraction being the first to be eluted, at alkaline pH, from Nuclepore filters loaded with lysed cells;
2) dose-effect curves (between 0 and 4 Grays) show that generation, by x-rays, of single strand breaks occurs with a higher frequency in the DNA of proliferating cells than in the DNA of differentiating cells, with little or no difference between committed cells (at day 6 after their transfer to the fusion medium) or fully differentiated (at day 8) myofibers.

The comparison between the phenotypic answer of the cells to radiation exposure (estimated from the extent of the inhibitory effect on cell fusion) and the evaluation (by the alkaline elution procedure) of the actual genomic damage and/or rearrangement, should allow a better understanding of the role played by chromatin rearrangement and/or DNA repair mechanisms in protecting the differentiating cells from x-rays damage.

As a further aspect of this problem, cellular differentiation was correlated to variations in site-specific or genome-wide DNA methylation. Two members of this group (D. Carotti and S. Scarpa) have collaborated in 1986 to experiments indicating that a transient hypomethylation (probably due to active removal of methyl groups), followed by remethylation, takes place during the first stages of differentiation of Friend erythroleukemia cells. In order to investigate if active demethylation can indeed be

considered to be a general phenomenon linked to cell differentiation, and to verify whether it modified the sensitivity to ionizing radiations of the differentiating cells, the level of DNA methylation was measured at various times during the differentiative process of cultured myoblasts. To this purpose, L5 myoblasts in the replicative status (i.e. with 10% foetal calf serum in the culture medium) were labeled for 40h with ^{14}C-uridine. After induction of differentiation by cell transfer to the "fusion medium", the DNA from increasingly "older" cultures was isolated and hydrolyzed to bases, and the extent of cytosine methylation was measured by HPLC. No significant variation of the methylcytosine-to-cytosine ratio could sofar be evidenced under these experimental conditions. Even addition of 3-deazaadenosine, known to perturbate S-adenosylmethionine metabolism, was unable to produce appreciable changes in the overall levels of DNA methylation. We are at present investigating whether, in the myoblast system, these changes can be shown to occur in more restricted portions of the genome, in particular in those regions which, from the alkaline elution experiments, appear to be more susceptible to single-strand breaks upon differentiation and/or exposure to x-rays.

This problem was also tackled in CHO cells by investigating how the increased frequency of sister chromatid exchange, caused by the two hypomethylating drugs L-ethionine and 5-azacytidine, was actually correlated, through subsequent cell cycles, to the 5-methylcytosine content of nuclear DNA. It was found that, while there was an excellent correlation in the first cycles after addition and removal of the drug, overall DNA methylation levels returned to "normal" values between the 10th and the 16th cycle after removal of the hypomethylating drug, despite the persistence of a high frequency of sister chromatid exchange.

Investigations on the *in vitro* susceptibility to methylation of natural genomic sequences differing in CpG frequency have also been continued. As a consequence of our previous results with some cloned murine CpG-rich "islands" (indicated as "pH9.2" and "pI.9.2"), a more detailed analysis was performed -- using the same experimental approach, i.e. by determining, through digestion with restriction endonucleases, to which extent *in vitro* methylation with added DNA-methyltransferase had indeed been effective on specific cytosine moieties -- on other cloned sequences, i.e. on two other plasmids carrying either a human X-linked gene or the human β-globin gene). Similar results were obtained for every sequence tested, without any interference by pre-existing methylation levels: DNA regions having an average of at least one CpG per 11 nucleotides (this ratio being a typical frequence in a CpG-rich island) underwent methylation less efficiently than regions having a lower CpG frequency; isolated CpG's, generally considered as less favorable substrates, became instead methylated to a higher extent than clustered ones in our *in vitro* system. These results show a peculiarity of DNA methyltransferase activity which can account for the "physiological" *in vivo* situation, in which only clustered CpG's are unmethylated: also *in vitro*, in fact, enzymatic methylation of these clustered CpG's was somewhat unfavoured -- although in the latter case, at variance with the *in vivo* situation, it was not totally prevented. This discrepancy between the *in vivo* situation and the *in vitro* results suggests that, *in vivo*, interaction with other chromatin component(s) can also play a role in preventing methylation of clustered CpG's -- but no direct experimental evidence supporting this hypothesis has sofar been obtained. Experimental data concerning the influence of chromatin structure (and of its different components) on the activity of eukaryotic DNA-methyltransferase have instead shown that, at least in human placenta chromatin, there are some tightly-bound components which strongly stimulate, *in vitro*, the enzyme-catalysed transfer of methyl groups to DNA cytosine moieties. It was also found, as an *in vivo* counterpart of this phenomenon, that, in chromatin loops, there are some DNA segments characterized by a high methylation level and by a strong association with some "tightly-bound proteins". As a further aspect of these investigations, it was verified -- using a method which had previously been developed within this project -- whether the DNA-methyltransferase-catalysed transfer of methyl groups from S-adenosylmethionine to the 5 position of cytosine moieties of DNA was in exact stoichiometry with the liberation of the hydrogen atom from this same position of the cytosine moieties -- since the latter event could possibly occur also by an enzyme-catalysed exchange reaction not necessarily linked to actual DNA methylation. While a 1:1 stoichiometry was indeed shown to hold for the "hemimethylation" reaction, equality between the two measures was not found, sofar, in the "de novo" methylation reaction.

IV. Objectives for the next reporting period:

In order to overcome the progressive loss of cell synchronization in the cultures of differentiating myoblasts, we plan to pre-label the cells in their DNA, so as to establish whether the radiation damage really occurs selectively in the non-differentiating fraction of the overall myoblast population, whether protection against x-radiation by DZA/HCY can be due to more efficient repair mechanisms, and to which extent enzymatic methylation of the cytosine moieties modulates the repair process. We shall also study the different chromatin and/or DNA fractions to determine whether some regions are more susceptible to single-strand breaks and/or to methylation/demethylation processes, both as an effect of radiation damage and of cell differentiation. In particular, the different fractions obtained by the DNA alkaline elution technique will be analysed for their 5-methylcytosine content -- as compared to the occurrence of single-strand breaks -- in order to study the kinetics of chromatin rearrangement and the dose-effect curves during the commitment period. Further investigation shall also deal with the identification and localization within chromatin structure -- taking into special consideration the chromatin regions which differ in their susceptibility to single-strand breaks -- of the chromatin components which regulate the activity of DNA methyltransferase.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. P. Lavia: Centro di Genetica Evoluzionistica del C.N.R., c/o Dip. di Genetica e Biologia Molecolare, Università di Roma "La Sapienza", Roma (Italy).

Dr. P. Perticone: Dip. di Genetica e Biologia Molecolare, Università di Roma "La Sapienza", Roma (Italy).

Prof. F. Cacace and Dr. M. Attinà: Dip. di Chimica e Tecnologie delle Sostanze Farmacologicamente Attive, Università di Roma "La Sapienza", Roma (Italy).

Dr. O. Sapora and Dr. M.A. Tabocchini: Istituto Superiore di Sanità, Viale Regina Elena 299, Roma (Italy).

Dr. D. Tonlulo: Istituto Internazionale di Genetica e di Biofisica del C.N.R., Napoli (Italy).

Dr. H. P. Saluz: Friedrich-Miescher Institut, Basel (Switzerland).

VI. Publications:

1. PUBLICATIONS IN SCIENTIFIC JOURNALS, MONOGRAPHS

1) P.Calafa, S. Mastrantonio, F. Cacace, M. Attinà, M. Rispoli, R. Strom: "Localization, in human placenta, of the tightly-bound form of DNA methylase in the third level of chromatin organization". Biochim. Biophys. Acta 951, 191-200 (1988).

2) P.Calafa, S. Mastrantonio, M. Attinà, M. Rispoli, A. Reale, R. Strom: "Do tightly bound chromatin proteins play a role in DNA methylation?". Biochem.Intern. 17, 863-875 (1988).

3) P. Calafa, A. Tomassetti, S. Mastrantonio, A. Reale, M. Spinelli, R. Strom: "Tightly bound non-histone proteins in different nucleosome-like subpopulations from pig kidney chromatin". Cell Biochem. Funct. 6, 39-45 (1988).

4) S. Scarpa, O. Sapora, M. A. Tabocchini, L. Di Renzo, M. Bigioni, S. Pazzaglia, F. Palitti, D. Carotti, R. Strom: "Effect of X-rays on the differentiation of L5 myoblast cell line". Ital. J. Biochem, accepted for publication (1988).

5) G. Ricci, G. Del Boccio, A. Perelli, A. Aceto, E. P. Whitehead, G. Federici: "Non equivalence of the two subunits of horse erythrocyte glutathione transferase in their reaction with sulphydryl reagents". J. Biol. Chem., accepted for publication (1988).

6) E.P. Whitehead, B. Taddeo, E. Stampeggiani, F. Palitti, D. Carotti: "Measurement of DNA methylase activity by tritium release from DNA cytosine". Cell Biophysics, accepted for publication (1988).

2. SHORT COMMUNICATIONS, THESES, INTERNAL REPORTS

1) S. Scarpa, O. Sapora, M. A. Tabocchini, L. Di Renzo, M. Bigioni, S. Pazzaglia, F. Palitti, D. Carotti, R. Strom: "X-rays effect on the differentiation of L5 myoblast cell line". Comm. 26° Meeting Europ. Tissue Culture Society (Gent), Aug.30-Sept.2, 1988.

2) F. Palitti, D. Carotti, S. Scarpa, R. Strom: "DNA metiltransferasi nel differenziamento di mioblasti di ratto". Com. 34° Congr. Soc. It. Biochimica (Padova), 2-4 Ott. (1988).

3) S. Scarpa, O. Sapora, M. A. Tabocchini, L. Di Renzo, M. Bigioni, S. Pazzaglia, F. Palitti, D. Carotti, R. Strom: "Effetto dei raggi X sul differenziamento della linea L5 di mioblasti di ratto". Com. 34° Congr. Soc. It. Biochimica (Padova), 2-4 Ott. 1988.

4) P. Perticone, F. Palitti, R. Cozzi, M. D'Erme, R. Bono: "Level of DNA methylation and sister chromatid exchanges in chinese hamster ovary cells in vitro". Atti Assoc. Genetica Italiana vol. XXXIV (Perugia), 24-26 Ott. 1988.

5) F. Palitti, R. Cozzi, M. D'Erme, P. Perticone: "Relation between SCE induction and methylation level in CHO cells treated with demethylating agents". Comm. 16° Intern. Congr. Genetics (Toronto), Aug. 20-22, 1988.

6) F. Palitti, R. Cozzi, M. D'Erme, P. Perticone: "Methylation level and damage in mammalian cells". Comm. 14° Intern. Congr. Biochemistry (Prague), July 10-15, 1988.

7) M. D'Erme, A. Reale, G. D'Andrea, R. Strom, P. Calafa: "Presence and distribution of tightly-bound histones in human placenta chromatin". Comm. 14° Intern. Congr. Biochemistry (Prague), July 10-15, 1988.

8) P. Calafa, M. Rispoli, S. Mastrantonio, A. Reale, R. Strom: "Regulation of DNA methylation by tightly-bound chromatin proteins". Comm. 14° Intern. Congr. Biochemistry (Prague), July 10-15, 1988.

9) E.P. Whitehead: "A simple proof of the King - Altman algorithm for steady state enzyme kinetics equation". Comm. Meeting Biochemical Society (London), Dec. 19-22, 1988.

10) E.P. Whitehead, R. Nucci, C.A. Rala, C. Vaccaro, M. Rossi: "Hill ratios equal binding ratios of allosteric enzyme effectors: dCMP aminohydrolase". Comm Meeting Biochemical Society (London), Dec. 19-22, 1988.

11) E.P. Whitehead, B. Taddeo, E. Stampeggiani; F. Palitti, D. Carotti: "A simple assay for DNA methylase". Comm. Meeting Biochemical Society (London), Dec. 19-22, 1988.

12) "DNA-Metiltransferasi e livelli di metilazione del DNA nella cromatina di placenta umana". Exp.Thesis by V. Quaresima, University of Roma "La Sapienza" (Faculty of Pharmacy), 1988 (P. Calafa, relatore).

13) "Proteine cromatiniche istoniche strettamente legate al DNA: localizzazione e caratterizzazione". Exp. thesis by P. Pugnaloni, University of Roma "La Sapienza" (Faculty of Pharmacy), 1988 (P. Calafa, relatore).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-177-I

Università degli Studi di Milano
Via Festa del Perdono, 7
I - 20122 Milano

Head(s) of research team(s) [name(s) and address(es)]:

Prof. L. Tallone Lombardi
Dipartimento di Fisica
Università degli Studi di Milano
Via Celoria, 16
I - 20133 Milano

Telephone number: 02-2392/235

Title of the research contract:

Radiation carcinogenesis in vitro.

List of projects:

1. Radiation carcinogenesis in vitro.

Title of the project no.:

Radiation carcinogenesis in vitro.

Head(s) of project:

L. Tallone Lombardi

Scientific staff:

D. Bettega, P. Calzolari, A. Ghidoni, A. Ottolenghi

I. Objectives of the project:

Determination of the dose effect relationship for cell transormation in C3H10T1/2 exposed to α particles (from ^{244}Cm) in the dose range between 0.01 and 2 Gy at two dose rates, 5×10^{-3} Gy/min and 0.1 Gy/min. Study of dose protraction effects and repair processes.

II. Objectives for the reporting period:

Preparation of the apparatus for cells irradiation with alpha particles. Optimization of the culture techniques of C3H10T1/2 cells in order to minimize the spontaneous transformation frequency. Preliminary runs.

Analysis of the growth kinetics of C3H10T1/2 cells exposed to various doses of X-rays. Analysis of radiobiological and cytogenetic characteristics of radiation transformed lines.

III. Progress achieved:

Our main activities in 1988 centred on continuing the in vitro studies of dose-response relationships for transformation induced by ionizing radiations of various LETs.

The apparatus for irradiation with alpha particles has been completed. The irradiation chamber is a stainless steel cylinder, 16 *cm* in height and 14 *cm* in diameter divided into two compartments. A 2 *cm* diameter Curium 244 alpha particle source is located at the bottom of the chamber. The total activity is 42 MBq. A set of externally controlled collimators of various diameters, are used to limit the dimension of the source and consequently to vary the dose rate. The alpha particles traverse a path of 6.9 *cm* of helium at atmospheric pressure and a window of 1.5 micron of mylar + 1 *mm* of air before entering the base of the culture dish. Cells are cultured on a 3 *cm* diameter, 1.5 thick mylar foil sealed with araldite to a pyrex ring. Using these supports no difference has been found in growth rate and plating efficiency as compared with cells cultured on conventional Petri-dishes. With this arrangement, we have also succeeded in keeping spontaneous transformation frequency less than 10^{-5} transformed per surviving cell. A special thermostatic chamber has been built to keep the cells at $37°C$ and in an atmosphere with 2.5% CO_2 during low dose-rate exposures. The energy of the alpha particles incident on the cells is 4 Mev and their LET 108 $KeV/\mu m$ in unit density tissue. The homogeneity of the beam over the area of the samples has been measured by CR39 plastic track detectors. The flux of etched holes was uniform, variation over the entire area being less than 10%. Dose measurements were performed using a parallel plate ionization chamber one plate of which is made of 1.5 micron thick mylar foil coated with a thin Al layer. C3H10T1 2 cells were irradiated with alpha particles at a dose rate of 0.1 $Gy\ min$. Survival curves in the range between 0.01 and 2 Gy were determined. This curve is a pure exponential with mean lethal dose value of $0.61 \pm 0.02\ Gy$. Preliminary data on transformation frequency in the same dose interval were also obtained. Analysis of the data is in progress.

Analysis of the growth curves and size of the colonies of C3H10T1 2 cells

exposed to various doses of low LET radiation has been completed. Cell density at confluence was 3.3×10^4 $cells/cm^2$. The initial division delay was very small; in the first 15 h the increase in the cell number was essentially the same at all doses. The temporal dependence of the growth properties of surviving and non surviving cells was studied. The following conclusions were drawn: growth curves are influenced by the non-survivor progeny up to 150, 200, and 250 h from irradiation at 3, 5, and 7 Gy respectively; at longer times, the population can be considered as consisting of surviving progeny. Surviving cells grow with a constant rate $a = 0.029\,h^{-1}$; non-surviving cells have a growth rate equal to a, but they lose their ability to divide with a rate per unit of dose $b = 0.0041\ h^{-1}Gy^{-1}$.

Morphologically transformed type II and III foci arising on a monolayer of irradiated C3H10T1/2 were isolated and subcultured into transformed lines. Behaviours after irradiation and cytogenetic characteristics of the transformed lines were analyzed and compared with those of the untransformed control cells. Transformed cells have a higher plating efficiency in soft agarose medium and in low concentration fetal calf serum,a wider chromosome number distribution. Transformed and untransformed populations are able to repair sublethal radiation damage but in the case of the transformed this repair occurs more slowly. No significative difference was found in the growth-rate in the exponential phase, but the density at the monolayer is about ten times greater in the transformed. No significative chromosome rearrangements were found in the reconstructed karyotypes. Southern blotting analysis indicates that none of the oncogenes tested, (ki-ras, Ha-ras, N-ras, c-myc, c-myb, c-abl) are amplified in these cells.

IV. Objectives for the next reporting period:

Determination of the oncogenic transformation frequency in C3H10T1 2 exposed to 4 *MeV* α particles in the dose range between 0.01 and 2 *Gy* at two dose rate values. Determination of RBE by comparison with data obtained with 220 *KVp* X-rays in the same experimental conditions.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

- Bettega D., Calzolari P., Ottolenghi A., Tallone Lombardi L., *Growth Kinetics of C3H10T1/2 Cells Exposed to Low LET Radiation*, International Journal of Radiation Biology, accepted for publication, 1988.
- Bettega D., Calzolari P., Ottolenghi A., Rimoldi E., Tallone Lombardi L., *Cell Density Dependence of Transformation Frequencies in C3H10T1 2 Cells Exposed to X-Rays*, submitted to International Journal of Radiation Biology, 1988.
- Privitera E., Mosna G., Sala E., Ghidoni A., Bettega D., Calzolari P., Ottolenghi A., Tallone Lombardi L., *A Cytogenetic, Molecular and Radiobiological Study of Five Transformed C3H10T1 2 Lines, One Characterized by Amplified Genetic Material*, submitted to Carcinogenesis, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-091-D

Kernforschungszentrum
Karlsruhe GmbH
Postfach 3640
D - 7500 Karlsruhe 1

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. D.M. Taylor
Kernforschungszentrum Karlsruhe
Inst.f.Genetik u.Toxikol.v.Spaltst.
Postfach 3640
D - 7500 Karlsruhe 1

Telephone number: 7247-82 4482

Title of the research contract:

The fractionation and speciation of plutonium and other actinide elements in vivo.

List of projects:

1. The fractionation and speciation of plutonium and other actinide elements in vivo.

Title of the project no.: BI6-D-091-D
The fractionation and speciation of plutonium and other actinide elements in vivo.

Head(s) of project: Prof Dr D. M. Taylor

Scientific staff:

Prof Dr V. Volf	Dr W. Rau
Prof Dr A. Seidel	L. Yule
Dr F. Planas Bohne	B. Köhler
Dr W. G. Thies	E. A. Davies

I. Objectives of the project:

1. To study the chemical forms in which plutonium and other actinides are present in the blood plasma, in intracellular structures, such as lysosomes, and in the various compartments of the gastrointestinal tract.

2. To elucidate the mechanisms of transfer across plasma and mucosal membranes and to identify factors which may influence the transfer.

II. Objectives for the reporting period:

1. Continuation of the studies of the speciation and fractionation of actinides in the improved simulated gastrointestinal tract model.

2. Spectroscopic and chromatographic studies of the binding of actinide and lanthanide elements to transferrin and other serum proteins.

3. Feasibility study for the introduction of computer simulation methods for the study of metal speciation in the human gastrointestinal tract and in the human food chain.

III. Progress achieved:

1. SPECIATION OF ACTINIDES IN THE SIMULATED HUMAN GASTROINTESTINAL TRACT

The studies using a simple in vitro model system were continued using plutonium-238. The work was concentrated on an improved human duodenal system, as this region is assumed to be of most importance in relation to actinide absorption into the systemic circulation. With a more refined model, studies of the fractionation and speciation of plutonium in the simulated system were carried out at a pH of about 6 and in the presence of nitrate, citrate or orange juice. Results, using Sephadex gel-chromatography, ion-exchange chromatography, electrophoresis and solvent extraction, showed that in the presence of nitrate only 17% of the plutonium remained in soluble form and of this only about 0.1% was present as a neutral, presumably absorbable species. With citrate and orange juice 70 to 80% of the total plutonium remained in soluble form, but again only about 0.1% of this soluble material was in the form of neutral complexes. Computer simulation studies of the speciation of plutonium in the duodenum in the presence of citrate, carried out in collaboration with Dr J. R. Duffield of the University of Wales College at Cardiff, have also indicated that the expected fraction of uncharged soluble species would be about 0.1%. This value of 0.1% is of the same order as the observed absorption of plutonium in human subjects. Current work is concerned with the further refinement of the system and the identification of endogenous and exogenous factors in the gastrointestinal tract which may influence the proportion of uncharged soluble actinide species and hence the absorption of the elements into the systemic circulation.

2. SPECTROSCOPIC AND CHROMATOGRAPHIC STUDIES OF THE BINDING OF LANTHANIDE AND ACTINIDE ELEMENTS TO TRANSFERRIN.

Transferrin is the principal iron transport protein in mammalian blood serum, but numerous in vivo studies have shown that many non-physiological metals, especially the actinides are transported in the blood as transferrin complexes. The physiological behaviour of these "foreign metal" transferrin complexes differs from that of iron-transferrin, possibly due to conformational differences between the various complexes. To study further the nature of the binding of foreign metals to transferrin, the binding of the lanthanide ytterbium to rat serum in vivo and to horse serum in vitro was investigated. Sequential chromatography on Sephadex G200 and DEAE Sepharose showed that in both rat and horse serum transferrin was the principal binding protein for ytterbium. This binding was confirmed by further chromatography on Blue Sepharose-CL-6B and by displacement of the ytterbium by saturating the protein with iron. The binding appeared to be relatively weak, more similar to that of americium or curium than to the strong binding of plutonium or thorium.
UV difference spectroscopy showed that, like iron, plutonium and thorium, two atoms of ytterbium were bound per molecule of transferrin
The application of speciation computation methods permitted the calculation of a minimum value for the formation constant, log β, of the ytterbium-transferrin complex of about 12; some 10 to 15 orders of magnitude less than that of the iron-transferrin or Pu(IV) complexes.

In other studies the ability of several potential new chelators to remove plutonium from transferrin in vitro were investigated. Studies with LICAM(C) indicated that the ability of this compound to release transferrin decreased sharply below pH 7 and that at pH values between 5 and 6 LICAM(C) led to enhanced binding to transferrin, perhaps through the formation of ternary plutonium-LICAM(C)-transferrin complexes. A new quinolinic acid derivative, synthesised by Chinese scientists, was found to show valuable activity in vitro and in vivo.

3. COMPUTER SIMULATION METHODS FOR THE STUDY OF METAL SPECIATION

In collaboration with the School of Chemistry and Applied Chemistry of the University of Wales College at Cardiff work was begun on the introduction of computer simulation methods for the calculation of metal speciation in biological systems such as the gastrointestinal tract, blood plasma and in other biological situations such as contaminated wounds. Plans were made for the development not only of the relevant computer models but also for the necessary experimental methods for the validation of the models. A new Micro-VAX computer was delivered at the end of the year and is now being brought into service; considerable work was invested in the examination of a number of potential experimental methods which can help to validate the computer models.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. National Radiological Protection Board, Chilton, Oxon, UK
2. IPSN, CEA, Fontenay aux Roses, France
3. School of Chemistry and Applied Chemistry, University of Wales College at Cardiff, Cardiff, Wales, UK
4. Radiobiology Department SCK/CEN Mol, Belgien.
5. European Late Effects Project Group (EULEP)
6. Department of Haematology, Royal Free Hospital School of Medicine, University of London, London, UK

V. Publications:

Planas-Bohne, F., Duffield, J.R.. Factors influencing the uptake of iron and plutonium into cells. Int. J. Radiat. Biol. 53, 489-500, 1988.

Schuppler, U., Planas-Bohne, F., Taylor, D. M.. Biochemical binding and distribution of protactinium-233 in the rat. Int. J. Radiat. Biol. 53, 457-466, 1988.

Taylor, D. M.. Can in vitro methods reliably predict the efficacy invivo of new chelators? In: Chelating Agents in Pharmacology, Toxicology and Therapeutics, Eds. P. Sobotka and V. Eybl. Pilzen Medical Report, Suppl. 56 pp 11-12, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-184-IRL

Trinity College
IRL - Dublin 2

Head(s) of research team(s) [name(s) and address(es)]:

Dr. K.F. Tipton
Department of Biochemistry
Trinity College
IRL - Dublin 2

Dr. C. Mothersill
St. Lukes Hospital
Highfield Road
Rathgar
IRL - Dublin 6

Telephone number: 01-772941

Title of the research contract:

Interaction between radiation and environmental carcinogens, studies on human cells in vitro.

List of projects:

1. A study of the transformation of primary human cell cultures by radiation and radiation in combination with environmental mutagens.

Title of the project no.:

A study of the transformation of primary human cell cultures by radiation and radiation in combination with environmental mutagens

Head(s) of project: Dr K. F. Tipton
Dr C. B. Seymour
Dr C. Mothersill

Scientific staff: Dr K. F. Tipton
Dr C. Seymour
Dr C. Mothersill
Ms A. Cusack
Mr J. O'Rourke

I. Objectives of the project:

(1) To use primary cultures of human tissues to study the interaction between radiation and known or suspected environmental carcinogens, promotors, mutagens or toxins.

(2) To design model systems where a carcinogen's effect on its in vivo target organ can be studied in relevant systems in vitro.

(3) To screen treated cells for changes in ultrastructure or isoenzyme profile in addition to conventional transformation endpoints which often have to be studied in specialised cell lines or rodent models.

II. Objectives for the reporting period:

(1) To continue to develop a model epithelial culture system capable of showing quantitative changes following exposure to known carcinogens and radiation.

(2) To study the mechanism of the interaction between nitrosamines, polycyclic hydrocarbons and radiation in releasing target epithelial cells from growth control.

(3) To continue to develop immunocytochemical techniques which will detect oncogene activation and changes in proliferative fraction in exposed cells.

(4) To continue attempts to quantify the changes in LDH, cytokeratins and GST in explants treated with radiation and carcinogens.

(5) To study the effect of promotors on the population of cells showing increased rates of proliferation after carcinogen treatment.

(6) To use epidemiological data for cancer mortality to identify high risk areas for different cancers where synergistic factors could be involved

III. Progress achieved:

Methodology

The methodology used for the associated contract (B16 092 IRL) was used for most of these studies. Cancer mortality statistics were obtained and standardised mortality ratios (SMRs) calculated for each administrative county in Ireland. Maps for each cancer were then constructed. Data concerning environmentally important risk factors such as high radon areas can easily be overlaid in the form of transparancies. These data are in the process of being accumulated.

Results

(1) As noted in the report for 1987, the most striking and consistent effect of addition of a variety of carcinogens to cultures of normal epithelial tissues prior to low dose is a considerably increased area of cellular outgrowth from the parent explant after 1-2 weeks. This effect was investigated in detail in oesophageal and urothelial tissue using very specific combinations of nitrosamines and radiation - peak stimulation in oesophageal mucosa occurred at 0.05 µg/ml N ethyl nitrosamine in combination with 5 Gy gamma rays. Quantitative analysis of the cultures revealed an overall increase in the number of proliferating epithelial cells plus a specific and very large increase in cells which have an ultrastructure suggestive of squamous cell carcinoma cells and are positive both for epithelial and endothelial cell markers. These cells grow over the monolayer of epithelial cells and are the subject of intense investigation at present.

(2) Attempts to study possible mechanisms of interaction between various carcinogens have focussed on the possibility that radiation activates the carcinogens, making them more potent. Support for this suggestion comes from the observation that the carcinogen must be present before irradiation in order for increased proliferation to occur. The production of electrophilic species by the radiation is a likely mechanism.

(3) Considerable effort went into the development and application of techniques to identify cytokeratins associated with malignancy. Because of the small numbers of cells available it was necessary to pool cultures to get any results. By pooling 10 cultures for each gel it was possible to extract a sufficient amount of the intermediate filament fraction to detect separate bands corresponding to different cytokeratins using a microgel electrophoresis unit. The gel could be scanned and the different bands recorded. Because of the uncertainty concerning the initial amount of protein in the preparation, the technique is at present only capable of giving data concerning presence or absence of a keratin band or, at most, relative amounts of the bands within a single gel. The bands are clearly different following treatment with radiation alone or radiation + nitrosamines. Table 1 (at end of Discussion) shows a typical result. The malignancy associated cytokeratin 18 occurs in irradiated cultures and in those treated with nitrosamine and radiation but not in normal tissues or cultures from normal tissues.

(4) Addition of promotor (TPA) to nitrosamine and radiation treated cultures at levels in the range used in the literature (1-10 µM) produced a toxic effect. No enhancement of proliferation or extended lifespan was seen. The effect of lower levels of TPA and of different promotors is currently being investigated.

(5) Cancer mortality figures have been assembled from death statistics and hospital records. These have been analysed with respect to age, sex and site of cancer and a series of maps and tables have been produced. It is hoped to publish these figures separately (pre-print attached). Standardised mortality ratios (SMRs) have been produced for each administrative county. These allow areas of high cancer mortality to be established without merely reflecting the age of the population. Maps of possible risk factors (power plants, underlying geology, etc.) are being produced to allow areas of possible synergistic reaction to be identified.

Discussion

The overall aim of the project is to look at interactions between radiation and chemical carcinogens in culture and to accumulate epidemiological data so that the relevance to man of potentially synergistic interactions can be identified. Interactions between radiation and organ specific carcinogens have been found *in vitro* and their relevance to *in vivo* studies is now being examined. Since radiation may be acting by activating the carcinogens, it is possible that the effects could be relevant in cases where low level dietary or environmental carcinogen exposure was coupled with, for example, medical investigative or therapeutic radiation, occupational or accidental exposure. Data identifying possible areas of high environmental exposure to carcinogens being accumulated at present are being correlated with the cancer death information for Ireland, and should allow predictions to be tested.

Regarding the *in vitro* tests, the occurrence of considerable angiogenic activity and extensive proliferation and altered cytokeratin pattern of epithelial cells in cultures treated with radiation and carcinogens does suggest that the cultures are altered in a manner which could *in vivo* be associated with development of malignancy. Definitive tests for *in vitro* transformation of epithelial cells are still a matter for heated debate but it appears likely that conventional fibroblast endpoints are not sufficient.

TABLE 1

Sample	Dose	Keratins present
Normal control *in vitro*	-	4,5,6,8,14
Tumour " " "	-	4,5,6,8,14,18,19
Normal control *in vivo*	-	4,5,6,8,14
Tumour " " "	-	4,5,6,8,14,18
Normal *in vitro*	2.5 Gy	4,5,6,8,14,18,19
" " "	5.0 Gy	4,5,6,8,14,18
" " "	10.0 Gy	4,5,6,8,14,15,18,19
" " "	15.0 Gy	4,5,6,8,14,15,18,19
" " "	Nitrosamine + 5 Gy	4,5,6,8,14,18,19
" " "	Nitrosamine only	4,5,6,8,14

IV. Objectives for the next reporting period:

(1) To quantify increased proliferation of epithelial cells resulting from combined exposure to radiation and carcinogens.

(2) To quantify as far as possible the intermediate filament and biochemical changes.

(3) To investigate the problem of metabolic activation of carcinogens in culture and to see what role irradiation might have in this.

(4) To continue to study the effects of tumour promotors on the system.

(5) To continue epidemiological studies to identify risk factor combinations.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

N O N E

VI. Publications:

Listed with Contract B 16 092 IRL

Report: Cancer Mortality in the Republic of Ireland, 1976 - 1986
by C. Seymour and M. Moriarty

(Copy attached, to be published separately).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Rue Charles Lemaire, 1
B - 1160 Bruxelles

Contract no.: BI6-D-094-B

Head(s) of research team(s) [name(s) and address(es)]:

Dr. O. Vanderborght
Department of Radiobiology
CEN/SCK
Boeretang 200
B - 2400 Mol

Telephone number: 014-31.18.01

Title of the research contract:

Relation between decorporation of osteotropic alpha-emitters and long term prevention of radiation harm.

List of projects:

1. Incidence of osteosarcoma in mice in dependence of the level of incorporated radionuclides in bone as influenced by decorporation treatment.

Title of the project no.:

Incidence of osteosarcoma in mice in dependence of the level of incorporated radionuclides in bone as influenced by decorporation treatment.

Head(s) of project:

G. Schoeters

Scientific staff:

O.L.J. Vanderborght
G. Schoeters
S. Van Puymbroeck

I. Objectives of the project:

Two main problems are addressed in the project.

1. Why is a substantial decrease of body-burden by decorporation treatments (such as was obtained via alginate treatment in ^{226}Ra contaminated mice) not reflected on a decrease of bone cancers ? Is this a general phenomenon, or only due to some specific properties of ^{226}Ra ?
2. Can a substantial improvement in decorporation of radioactive heavy alkaline earth metals be obtained by changing the first successful approach of treatment.

II. Objectives for the reporting period:

1. Comparison of survival data and pathology data of the male C57BL mice contaminated via intraperitoneal injection with ^{241}Am and with ^{226}Ra (our previous experiment) and comparison with data on ^{241}Am toxicity in mammalia which are available in the literature.
2. Pathology of BALB/c mice contaminated with 2.1 kBq ^{241}Am. To assess further the suitability of the BALB/c mouse model for the study of bone tumours after decorporation therapy, the experiment is expanded : male mice and other injection levels of ^{241}Am are included as well.
3. Via the autoradiographs we want to investigate whether a chronic Na-alginate treatment translocates ^{225}Ra to sites which are critical for bone tumour induction (e.g. bone surfaces). This is one of the hypotheses to explain the lack of reduction of the number of bone tumours after an Na-alginate treatment which reduced the ^{226}Ra body-burden.

III. Progress achieved:

Methodology

1. Statistical analyses of survival data and of pathology findings from C57BL mice injected with either 0.5, 1.5, 4.7, 10.0 or 33 kBq ^{241}Am per mouse.
2. Histopathology of female BALB/c mice injected with 2.1 kBq ^{241}Am at 3 months of age. Expansion of the experiment with BALB/c mice : 70 male mice were injected intraperitoneally at an age of 10 weeks with 2.5 kBq ^{241}Am per mouse, while in addition 150 female mice were also injected with 0.8, 1.6 and 2.6 kBq ^{241}Am citrate. Control mice from each sexe were included in the experiment.
3. Interpretation of autoradiographs prepared at NRPB (Harwell, UK) via light microscopy.

Results

1. Bone tumour induction could be significantly attributed to ^{226}Ra and ^{241}Am, while only one out of 227 uncontaminated control mice developed osteosarcoma. In ^{226}Ra injected mice : 42 out of 44 bone tumours were malignant ; in ^{241}Am injected mice : 11 out of 18 bone tumours were malignant. The radiation doses of ^{226}Ra which we selected were situated at the upward part of the dose-response curve for osteosarcomas. The risk for osteosarcomas associated with average skeletal dose was similar for all the groups in which osteosarcomas developed and resulted in a risk coefficient of 21 mice with bone sarcoma per 10^4 mouse Gy. ^{241}Am injected mice only developed bone tumours at the two lower dose levels, at higher doses the animals died before bone tumours could develop. The risk coefficient for mice with bone tumours per 10^4 mouse Gy is at least 10 times higher after ^{241}Am injection than after ^{226}Ra injection. It may still be higher since we cannot exlude the possibility that at lower dose levels equal numbers or even more bone tumours could arise. The spine was the predominant site for the occurence of osteosarcomas. Nine out of 11 osteosarcomas occured in the spine after ^{241}Am injection, while only 21 out of 42 after ^{226}Ra injection. The difference between the two radionuclides, with respect to preferential location of the tumours in the spine, is statistically significant $0.05<p<0.10$. No other tumours could be significantly attributed to radionuclide injection in our mice. The spontaneous incidence of liver tumours and of lymphosarcomas is however high in our C57BL mice (resp. 16 % and 18 %).
2. 29 out of 64 female BALB/c mice, injected i.p. with 2.1 kBq ^{241}Am developed bone tumours. This is a much higher yield than was ever obtained in our previous experiments with male C57BL mice. The

spontaneous bone tumour incidence of these BALB/c mice is low. The disadvantage for the use of this strain is the high spontaneous occurence of lymphosarcoma (20 %).

3. Daily treatment with Na-alginate of mice injected with ^{226}Ra resulted after 6 months in a significantly decreased ^{226}Ra concentration per g bone ash in the lumbar vertebrae of treated mice ($p < 0.05$). Autoradiographs of lumbar vertebrae from alginate-treated and non-treated mice were prepared at NRPB (Harwell). Translocation of ^{226}Ra due to chronic decorporation treatment was not evident. Thus it is unlikely that due to mobilisation of ^{226}Ra from the bones by alginate, a redistribution occured so that more target cells close to bone surfaces are exposed.

Discussion

1. Comparison of toxic effects induced by ^{226}Ra and ^{241}Am in the same mouse strain (C57BL male mice) reveals that ^{241}Am is a more efficient carcinogen than ^{226}Ra. The toxicity ratio for bone tumour induction related to average skeletal dose is 10 and related to injected activity is 6.1. The toxicity ratio for reducing life span related to average skeletal dose is 15. Our male C57BL mice were sensitive to ^{241}Am with respect to reduction of life span, while the yield of bone tumours was low if compared with reported toxicity data on ^{241}Am in e.g. male CBA mice (Nilsson et al., 1976, Acta Radiol. Ther. Phys. Biol., 15).
2. The high number of mice dying with bone tumours after injection with 2.1 kBq ^{241}Am and the low yield of spontaneous bone tumours in these BALB/c mice make this mouse strain more promising to work with if effects of decorporation therapy need to be tested. Expansion of the injection doses will learn whether the yield of bone tumours which we obtained was maximum.
3. The lack of chronic alginate treatment for protection against bone tumour induction after ^{226}Ra injection in mice, despite a substantial reduction in body-burden, cannot be explained by enhanced translocation of ^{226}Ra to sites which are critical for bone tumour induction. Other possible explanations such as the need of rapid removal of radioactivity from critical sites need to be tested further. Chronic daily alginate treatment results in a decrease in body-burden which may proceed too slowly with time.

IV. Objectives for the next reporting period:

1. Continuation of the survival study with female BALB/c mice to test the suitability of this mouse strain as a model to work with in decorporation therapy studies.
2. Efforts will be committed to speed up removal of heavy alkaline earths with time after contamination. Chronic daily alginate treatment via diet after ^{85}Sr injection will be combined with alginate injection or with continuous infusion of alginate via osmotic pumps. If important changes in the removal rate of the radionuclides can be achieved, the efficiency of this treatment with respect to reduction of long-term radiation effects will be tested in BALB/c mice.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Pathology Section of Biology Department, SCK/CEN, B-2400 Mol, Belgium (Dr J.R. Maisin)
- Inst. für Biologie, Gesellschaft für Strahlen- und Umweltforschung, mbH, D-8042 Neuherberg, FRG (Dr A. Luz)
- National Radiation Protection Board, Chilton, Didcot, Oxfordshire OX11 ORQ, UK (Dr J. Stather)

VI. Publications:

G.E.R. Schoeters, O. Vanderborght : The effect of Zn-DTPA treatment on ^{241}Am removal from bones implanted in non-radiocontaminated mice. Health Physics, accepted.

G. Schoeters, O. Vanderborght : The comparative carcinogenicity of ^{241}Am versus ^{226}Ra in various mouse strains. Proc. 14th L.H. Gray Conference, Oxford, 11-15 September 1988.

O. Vanderborght : Future outline for EULEP decorporation programme 1990-1994.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-185-NL

Rijksuniversiteit Leiden
Stationsweg 46
P.O. Box 9505
NL - 2300 RA Leiden

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A.J. van der Eb
Department of Medical Biochemistry
Sylvius Laboratoria
P.O. Box 9503
NL - 2300 RA Leiden

Telephone number: 071-276115

Title of the research contract:

Studies on the molecular basis of radiation-induced carcinogenesis.

List of projects:

1. Induction of leukaemia in vivo.
2. Induction of oncogenic transformation in vitro.

Title of the project no.:

Induction of Leukemia *in vivo*
Contract nr. BI6D-185-NL

Head(s) of project:

Prof.Dr. A.J. van der Eb
Department of Medical Biochemistry
Sylvius Laboratoria P.O.Box 9503
2300 RA Leiden, The Netherlands

Scientific staff:

Dr. A.G.Jochemsen

I. Objectives of the project:

The objective of this project is to gain an understanding of the mechanism by which radiation (ionizing radiation or UV-light) causes cancer. As a model the induction of leukemia or lymphoma in mice is chosen. Bone marrow stem cells will be isolated and activated *myc* or *ras* oncogenes, belonging to different oncogene "complementation" groups, will be introduced using retroviral vectors. The infected cells will then be irradiated (ionizing radiation or UV) and transplanted back into mice. If these two types of radiation activate different pre-carcinogenic pathways, this could result in complementation of different oncogenes. Similar experiments can be carried out with primary mouse cell cultures which, after the various treatments, will be transplanted back, e.g. by subcutaneous injection.

II. Objectives for the reporting period:

Since our attempts to introduce genes into bone marrow stem cells have met with considerable difficulties not only in our lab but also elsewhere, it was decided to use transgenic mice harboring an activated oncogene as a source of bone marrow. From the laboratory of Dr. A. Berns (Cancer Institute, Amsterdam) there are now available mice strains transgenic for the pim-1 oncogene and for the c-*myc* gene, regulated by the H-2 promoter and the Ig^H promoter, respectively. Due to difficulties in breeding sufficient numbers of transgenic mice it has not yet been possible to carry out the proposed experiments involving bone marrow transplantation. Therefore, it was decided to concentrate first on the *in vitro* experiments (see project 2).

III. Progress achieved:

Due to the problems mentioned above in obtaining sufficient numbers of transgenic pim-1 mice it was not possible to start the experiments in which bone marrow is isolated and then treated with radiation, followed by transplantation into syngeneic animals. It was decided therefore to concentrate on *in vitro* epxeriments with cell cultures derived from transgenic mice.

IV. Objectives for the next reporting period:

In collaboration with Dr. A. Berns (Amsterdam) the planned in vivo experiments with bone marrow from transgenic mice will be started as soon as sufficient mice are available.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. A. Berns, Netherlands Cancer Institute, Amsterdam.

Dr. D. Valerio, The Radiobiological Institute TNO, Rijswijk.

Dr. G.R. Mohn, RIVM, Bilthoven University of Leiden.

VI. Publications:

Title of the project no.:
Induction of oncogenic transformation in vitro
Contract nr. BI6-D-185-NL

Head(s) of project:

Prof.Dr.A.J. van der Eb
Department of Medical Biochemistry
Sylvius Laboratories, P.O.Box 9503
2300 RA Leiden, The Netherlands

Scientific staff:

B. Klein

I. Objectives of the project:

The purpose of this project is basically the same as that of project 1 (research contract nr. BI6-D-185-NL), except that the model in which the primary effects of radiation are investigated is the in vitro transformation of cultured cells rather than carcinogenesis in vivo. An activated myc or ras oncogene will be introduced into primary rodent cell cultures or into suitable cell lines (mouse 10T½, rat 3Y1, rat-1). The cells will then be irradiated either with ionizing radiation or UV-light, and scored for (complete) morphological transformation.

II. Objectives for the reporting period:

The objective was to introduce an activated ras or myc oncogene into primary rodent cell cultures or established rodent cell lines, and subsequently treat the cells with ionizing or UV irradiation. The question then is which type of treatment can most efficiently cause a second transformation event which will lead to distinguishable morphological changes or alterations in growth behaviour. The method would possibly also lead to the development of a dependable and relatively cheap method for testing chemical or physical agents for their carcinogenic potential. The results obtained indicated that cells obtained by transfection of primary rat embryo cultures or established rat or mouse cell lines with ras or myc oncogenes were not suitable for the mentioned experiments because, 1) the primary cultures showed too high spontaneous transformation frequencies (see previous report), whereas the established cell lines 3Y1, rat-1 and 10T½ were found to be already weakly oncogenic before transfection with oncogenes. Therefore, it was decided to concentrate on cultures from mice transgenic for activated oncogenes.

III. Progress achieved:

1. Methodology

Since the attempts to use cell cultures transfected with activated oncogenes as starting cells for studies on radiation-induced in vitro transformation were unsuccessful due to high frequencies of spontaneous transformation, it was decided to abandon this approach, but to use cell cultures from transgenic mice harboring activated oncogenes. The approach is as follows: 7-8 days old transgenic mice were sacrificed and the kidneys were trypsinized and brought in culture. 2 days after seeding the cells ere irradiated with X-rays or UV. Part of the cultures was treated with the chemical mutagen ENU. The cultures were maintained for at least 6 weeks by changing the medium twice a week, and they were regularly checked for the appearance of morphologically altered colonies. Cultures from each category were also trypsinized and reseeded at lower cell density 1 day after treatment with the mutagens, or suspended in soft agar medium to score for anchorage-independent growth.

2,3. Results and Discussion

At the end of 1988 the following 2 transgenic mouse strains were available through a collaboration with Dr. Berns. (1) a strain harboring the mouse pim-1 oncogene regulated by the mouse H2 promoter (2) a strain harboring the mouse c-myc gene expressed from the immunoglobulin heavy chain (Ig_H) promoter. The Ig_H-c-myc mice are not suitable for our purpose since the Ig_H promoter will only be active in B-lymphocytes and not in fibroblast-type cultures. Therefore, experiments were carried out with cultures of baby mouse kidney (BMK) cells from mice transgenic for the pim-1 oncogene. The primary BMK cultures were irradiated with X-rays or UV-light or treated with ethyl nitroso-ureum (ENU). In none of the cultures morphological transformation was observed nor was any growth in soft agar detected. This negative result can be explained in two ways: (1) Studies by Dr. Berns and coworkers have recently shown that the transgene pim-1 was expressed at a very low level as mRNA in the kidneys of the mice, whereas pim-1 protein could hardly be detected. (2) Pim-1 may possibly only be active as an oncogene in cells from the lymphocytic lineage since the gene is found in an activated form only in T and B-cell lymphomas. If the former explanation is correct, in vitro transformation might only occur in cells in which the pim-1 gene is expressed. The experiments will be repeated therefore with a mouse strain showing high expression of the pim-1 gene in the kidneys. This strain was also generated by Dr. Berns. In addition, a new series of experiments is being planned using primary cultures from a mouse strain transgenic for the mouse c-myc gene (exons 2+3) regulated by the SV40 promoter. This viral promoter is known to be active in many different tissues. If transformed cell clones are obtained in cultures of one of the transgenic mice, attempts will be made to identify the second oncogene which is activated by the radiation treatment. It will also be examined whether this transformation assay can be developed into a method for testing oncogenic activity of chemical and physical agents. The pim-1 and H-2-prom-c-myc transgenic mice were kindly provided by Dr. A. Berns (Amsterdam). The SV40 promoter-c-myc transgenic mice will be provided by Dr. A.T. Natarajan (Leiden); he received the mice from Dr. I. Hausmann (Göttingen) who kindly allowed us to use these mice for our experiments.

IV. Objectives for the next reporting period:

see section 2,3 results

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. G. R. Mohn, RIVM, Bilthoven

Dr. D. Valerio, Radiobiological Institute TNO, Rijswijk

Dr. A. Berns, The Netherlands Cancer Institute, Amsterdam

VI. Publications:

J.L.M. van der Lubbe, H.J.M. Rosdorff, J.L. Bos and A.J. van der Eb. Activation of N-ras induced by ultraviolet irradiation in vitro. Oncogene Research 3, 9-20 (1988)

J.L.M. van der Lubbe, H.J.M. Rosdorff and A.J. van der Eb. Homologous recombination is not enhanced in UV-irradiated normal and repair-deficient human fibroblasts. Mutation res. 217, no.2. 153-161 1989

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Netherlands Energy Research
Foundation ECN
P.O. Box 1
NL - 1755 ZG Petten

Contract no.: BI6-D-203-NL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.F. van de Vate
Netherlands Energy Research
Foundation ECN
P.O. Box 1
NL - 1755 ZG Petten

Telephone number: 02246-4949

Title of the research contract:

Induction of myeloid leukaemia in mice by irradiation with fission neutrons as a function of dose-rate.

List of projects:

1. Induction of myeloid leukaemia in mice by irradiation with fission neutrons as a function of dose-rate.

Title of the project no.:

INDUCTION OF MYELOID LEUKEMIA IN CBA/H MICE BY IRRADIATION WITH FISSION NEUTRONS AS A FUNCTION OF DOSE-RATE

Head(s) of project:
Dr. J.F. van de Vate

Scientific staff:
Dr. R. Huiskamp and Drs. J.A.G. Davids

I. Objectives of the project:

Radiation with a low linear energy transfer (LET), such as gamma- or X-rays is carcinogenic in man and in experimental animals. Since the replacement of the T65 dosimetry system for atomic bomb survivors by the DS86 dosimetry system, data on man concerning the effects of high -LET neutron iradiation, are not available any more. Thus, risk estimations for neutron irradiation of man can only be based on experimental studies in animals and depend on dose-response relations and the relative biological effectiveness (RBE) of neutrons. An additional factor of considerable importance for risk estimations, is the influence of protraction or fractionation of the dose on the degree of the effect.

II. Objectives for the reporting period:

In general, the biological effects of low-LET radiation at low dose rate are less than after acute exposure. The influence of reduced dose rate with high-LET radiation is as yet far from clear. Hill et al. (1982,1984) reported enhanced neoplastic transformation in vitro at a reduced neutron dose rate but these experiments have been debated extensively. Corresponding studies on carcinogenesis in vivo after neutron irradiation have also produced conflicting results. In the present investigation, radiation-inducible acute myeloid leukemia in mice was used as a model for further investigation of this so-called inverse or reversed dose rate effect in radiation-induced carcinogenesis by high-LET radiation.

III. Progress achieved:

1. Methodology

Animals

Male mice of the inbred CBA/H strain given to the Petten laboratory by Dr R.H. Mole (Medical Research Council Radiobiology Unit,Harwell, UK) and maintained at Petten since 1981, were irradiated or sham-irradiated at the age of 15-20 weeks. The procedures of animal care have been described elsewhere (Davids et al., 1970).

Irradiation procedures

The fast fission neutron source used in these experiments was a ^{235}U converter in the biological irradiation facility in the Low Flux Reactor (LFR) of the Netherlands Energy Research Foundation (ECN) at Petten. The design of this exposure facility, the tissue dosimetry and neutron spectrometry have been described elsewhere (Davids et al., 1969). In brief, the animals were exposed bilaterally to fast fission neutrons at dose rates of 2, 10 or 100 mGy/min. The absorbed doses are given as neutron center-line doses and do not include the 9% gamma-ray contribution. The variation in neutron center-line doses over the 40 mouse positions in the exposure facility is within 2% of the mean value. The neutron energy spectrum has a mean value of 1.0 MeV and the trackaverage LET_∞ for the first collision recoil protons is about 57 keV per micron in tissue.

Experimental procedures

A few minutes before exposure, the animals were placed individually in thin-walled closely fitting polycarbonate tubes. The tubes with mice receiving irradiation at the same dose rate were fitted in styrofoam columns as described by Davids et al (1969). The animals were irradiated with 400 mGy fast fission neutrons at a specified dose rate. During the course of this investigation three identical experiments were carried out. Numbers of mice presented in the various tables or figures, represent the pooled number of mice analysed in these experiments. After the irradiation, animals were returned to their cages, 2-3 animals per cage, and were weighed weekly.

Each animal cage was examined daily for dead or affected mice. Ani-

mals which showed weight loss and developed a marked pallor of blood vessels and of the normally red area at the root of the claws and a noticable pale appearance of the paws, were examined hematologically. Moribund animals were deliberately killed. At the standard post-mortem examination for all animals, the tissues taken for miroscopic examination included kidney, liver, lungs, spleen, sternum and other visibly affected organs. Tissues were fixed in formalin, embedded in paraffin, and sectioned at 5 microns. The sections were stained with hematoxylin-eosin.

When an animal was suspected to have myeloid leukemia, part of the spleen was processed for transplantation into syngeneic recipients. In brief, spleen cell suspensions were made in tyrode solution by mincing and gently pressing through a nylon gauge filter (pore size, 200 microns) and subsequently syringing through a 25 gauge needle. Cell number was measured with a Coulter electronic cell counter. Cell viability was measured using the trypan-blue dye exclusion test. 1-10 $*10^6$ viable spleen cells were injected intravenously in syngeneic recipients. The recipients were thereafter monitored for a developing myeloid leukemia which usually occurred 40-90 days after inoculation. Mice surviving 300 days after inoculation were killed and examined to confirm the absence of myeloid leukemia. Each transplantable myeloid leukemia was first maintained by serial passages in syngeneic recipients, but later spleen cell suspensions were stored in liquid nitrogen using 10 % DMSO in Dulbecco's modified medium supplemented with 10 % fetal calf serum.

2. Results

During the course of these experiments, no outbreaks of epidemic disease or other untoward phenomena affecting animal survival were observed. Nine animals with severe post-mortem autolysis and 16 animals without histology were excluded from the subsequent analysis (Table I).

Myeloid leukemia in male CBA/H mice usually involved the spleen and liver causing a marked splenomegaly and hepatomegaly. Histologically, myeloid leukemia was evidenced by a replacement of normal bone marrow and a marked diffuse and focal infiltration of the liver by

abnormal myeloid leukemic cells (Fig. 1). Frequently, necrosis of bone marrow cells was observed in one or more compartments of the sternum, probably related to leukemic emboli as also observed in the lungs. Infiltration of the kidney (Fig. 2) and extra-medullary infiltration of sternal muscles by myeloid leukemia cells were usually also observed. Routinely, an attempt was made to confirm the diagnosis of myeloid leukemia by transplanting spleen cell suspensions from mice with suspected myeloid leukemia in syngeneic recipients. Of 18 attempts, 15 succeeded. In two cases of unsuccessful tranplantation, the animals turned out to have a lymphosarcoma and a tumor of unknown

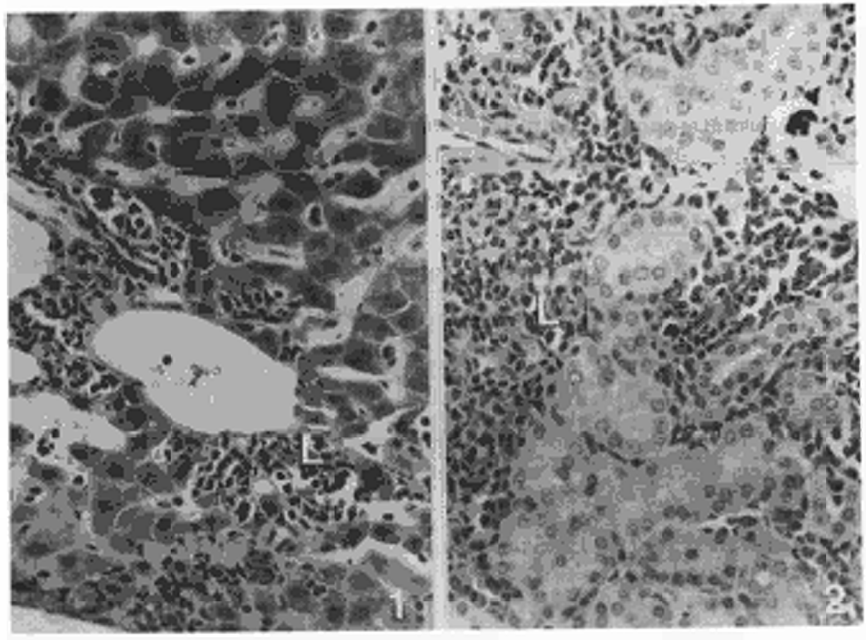

Fig.1. Liver in myeloid leukemia. Leukemic cells (L) are concentrated in the portal areas and diffusely distributed throughout the liver(x 360).
Fig.2. Infiltration of leukemic cells (L) in the kidneys(x 360).

origin, respectively. However, the third case was diagnosed as a true myeloid leukemia on histological criteria. As shown in Table I, myeloid leukemia was not observed in the 74 specific control animals used in this experiment and none has been seen in more than 1000 unirradiated or sham-irradiated male CBA/H mice examined before , during and since the experiment was carried out (Mole, 1986). In contrast, in each irradiated group 8 or more myeloid leukemias were observed. As shown in Table I, 29 myeloid leukemias were observed in the 258 mice given 400 mGy fast fission neutrons (11.2 %). The results of the present experiment show clearly that the observed myeloid leukemia frequencies are independent of dose rate since decreasing the dose rate from 100 mGy/min to 2 mGy/min did not cause a measurable change (Chi-square analysis, P> 0.80) in the myeloid leukemia frequency (Table 1).

TABLE I

Leukemia incidence in male CBA/H mice after 400 mGy fission neutron irradiation at different dose rates.

	Dose rate (mGy/min)			
	0.0	2.0	10.0	100.0
Number of mice irradiated:	88	90	91	88
Number of mice excluded:	14*	3*	2*	6*
Myeloid leukemia	0/74** (0.0)	10/87 (11.4)	11/89 (12.3)	8/82 (9.8)
Lymphosarcoma	1/74 (1.4)	1/87 (1.1)	3/89 (3.4)	4/82 (4.9)

*)Mice were excluded from the analysis because of either autolysis or no histology .

**)Numbers in parenthesis represent incidence expressed as percentages.

Besides myeloid leukemia, only one other type of murine leukemia occurred. Lymphosarcomas were seen in all experimental groups and at slightly higher freqencies in the higher dose rate groups (Table 1) . CBA/H mice normally show a high incidence(60-80 %) of hepatoma (Major, 1979). The distribution of hepatoma in irradiated and sham-irradiated groups hardly differed. Harderian gland tumors were not observed in the

74 sham-irradiated mice whereas only a small number of harderian gland tumors was observed in the irradiated groups. As shown in Fig.3 and

TABLE II

Mean survival time of male CBA/H mice after 400 mGy fission neutron irradiation at different dose rates

Dose rate (mGy/min)	Group size	Mean survival time (days)	St. Dev.
0	74	650	118
2	87	580	143
10	89	571	138
100	82	616	131

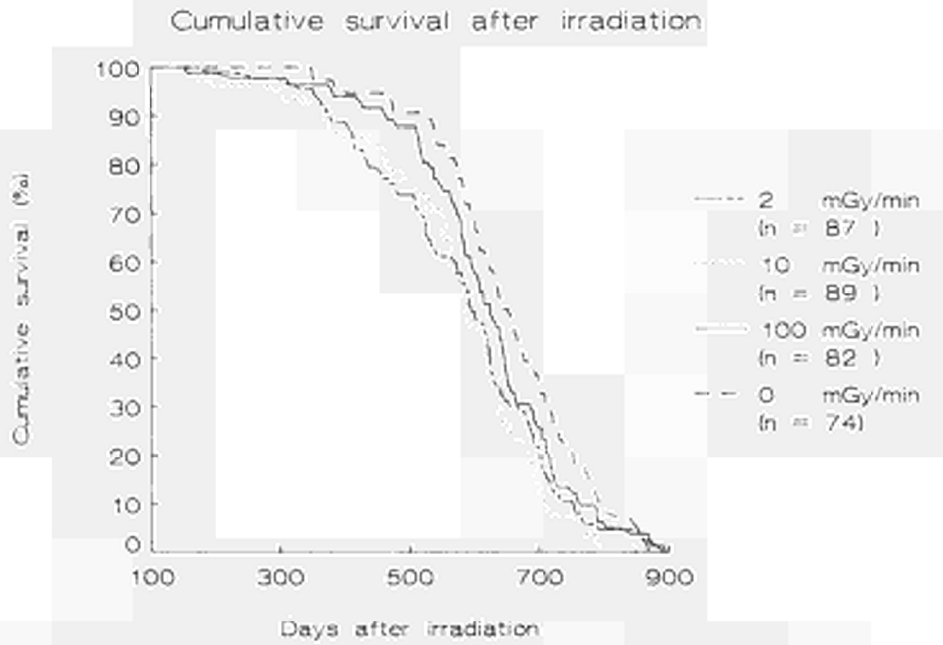

Fig.3: Cumulative survival of male CBA/H mice irradiated with 400 mGy fast fission neutrons at different dose rates.

Table II, the survival of each irradiated group was lower then that of the sham-irradiated control group. However, analysis of variance of all experimental groups showed no significant decrease ($P < 0.10$) in survival due to the fission neutron irradiation. Furthermore, comparison of each of the irradiated groups showed that survival was independent of the dose rate used (analysis of variance, $P < 0.50$).

DISCUSSION

The carcinogenic properties of densely ionising radiation are of basic relevance for radiological protection. Since the replacement of the T65 dosimetry system by the DS86 dosimetry system for the Japanese A-bomb survivors, information concerning the carcinogenic action of neutron radiation, such as 1 MeV fast fission neutrons, is no longer available in humans. Assessment of the neutron risk in man therefore depends on neutron induced carcinogenesis in experimental animals and on in vitro systems. In contrast to the tissue sparing effect of dose protraction generally observed with low-LET radiation, Hill et al. (1982, 1984, 1985) reported a marked enhanced neoplastic transformation of $C3H10T_{1/2}$ cells in vitro by JANUS reactor fission neutrons given at low dose rates or in fractionated exposures with fission neutrons vs high dose rate by a factor of about 8. On biophysical grounds, these observations are highly unexpected (Barendsen, 1985) and have been extensively discussed (Elkind & Hill, 1985; Burch & Chesters, 1986; Rossi & Kellerer, 1986) with as yet only tentative explanations. Experiments on cell transformation in vitro by high-LET alpha particles or neon ions or TRIGA reactor fission neutrons did not show enhancement of neoplastic transformation when the exposure was protracted (Hieber et al., 1987; Yang et al., 1986, Balcer-Kubiczek et al.,1988). In vivo studies on experimental carcinogenesis comparing protracted or fractionated high-LET irradiation with brief single exposures also produced conflicting results varying from reduction in effect (Ullrich, 1984), no change (Ullrich, 1984; Broerse et al., 1985; Little et al., 1985; Fry et al., 1985; Maisin et al., 1988), to enhancement of the investigated

radiation effect (Ullrich, 1984; Thomson et al., 1985; Lundgren et al., 1987).

In the present investigation, we used the radiation-induced acute myeloid leukemia model in male CBA/H mice and LFR fast fission neutrons. This model has a marked advantage that the natural myeloid leukemia incidence is extremely low. Moreover, acute myeloid leukemia is of special interest in radiological protection and the influence of protraction of low-LET gamma-rays on the leukemia induction in male CBA/H mice is closely similar to what has been observed in man after X-ray therapy for ankylosing spondylitis (Mole & Major, 1983). As shown in a previous investigation using LFR fission neutrons and male CBA/H mice (Mole & Davids, 1982), myeloid leukemia induction by fast fission neutron irradiation is linear with dose whereas the frequency of overt leukemia is less than the frequency of induction because survival of the transformed cells is dose dependent and proportional to exp(λ*dose). In this expression λ represents the retention of clonogenicity. Based on their dose-response model, 10.8 percent of the irradiated animals in the present experiment should have developed myeloid leukemia. This number corresponds very well with the observed myeloid leukemia frequencies (Table I). Furthermore, the present investigation clearly showed that protraction of the neutron dose has no measurable influence on the induced frequency of myeloid leukemia . Although only limited numbers of lymphosarcoma were observed, the incidence tended to increase with higher dose rates as has also been observed for murine ovarian tumors (Ullrich, 1984).

Although the survival in all three experimental irradiation groups was lowered in comparison with sham-irradiated animals (Table III; Fig.3), the decrease in survival after 0.400 Gy fast fission neutrons was not significant ($P < 0.10$). The main cause of death was hepatoma, a neoplasm observed in 70- 80 percent in both irradiated and sham-irradiated mice (Major, 1979; Mole & Davids, 1982). As also shown by Maisin et al. (1988), survival in the irradiated groups was independent of the dose rate. The latter results are in contrast with those reported by Thomson et al.(1982; 1985) on life shortening in mice after fission neutron irradiation. However, in these studies higher doses were used.

Summarizing, the present investigation using the radiation induced myeloid leukemia model clearly showed that reducing the dose rate of high-LET fast fission neutrons had no influence on the incidence of myeloid leukemia in male CBA/H mice. In addition, reducing the dose rate had no effect on survival of irradiated mice. In contrast, a higher incidence of lymphosarcomas was observed at higher dose rates.

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IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. R.H. Mole, formerly Director Medical Research Council, Radiobiology Unit, Harwell, U.K., present adress: Heath Barrows, Bayworth Lane, Boar's Hill, Oxford OX1 5DF, U.K.

V. Publications:

Mole, R.H. and Davids, J.A.G., Induction of myeloid leukemia and other tumors in mice by irradiation with fission neutrons. In : Neutron Carcinogenesis, J.J. Broerse and G.B. Gerber, Eds., p. 31-42, Rapport EUR-8084 EN (1982).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Welsh National School of Medicine
Heath Park
GB - Cardiff CF4 4XN

Contract no.: BI6-D-097-UK

Head(s) of research team(s) [name(s) and address(es)]:

Prof. E.D. Williams
Department of Pathology
Welsh National School of Medicine
Heath Park
GB - Cardiff CF4 4XN

Telephone number: 0222-755944 (Ext. 2700)

Title of the research contract:

Studies of the mechanism and prevention of low dose radiation carcinogenesis of the thyroid.

List of projects:

1. Studies of the mechanism and prevention of low dose radiation carcinogenesis of the thyroid.

Title of the project no.: B16-D-097-UK

Head(s) of project: Professor E.D. Williams

Scientific staff:

Dr. V. Wynford-Thomas : Post-Doctorate Research Officer
Miss K. Horler : Research Officer
Dr. N. Williams : Clinical Lecturer

I. Objectives of the project:

To study the quantitative relationship between low dose radiation and the development of thyroid tumours in the rat and to study the role of the growth stimulatory effect of TSH on tumour development and the value of TSH suppression in the prevention of radiation induced thyroid tumours.

II. Objectives for the reporting period:

To study the relationship between low dose radiation of the thyroid and the number of tumours induced in the goitrogen model, and to study the effect of temporary or permanent interruption of the growth stimulus on carcinogenesis.

III. Progress achieved:

1. Methodology:

Male Wistar rats were used for all experiments, radiation was administered in the form of external X-rays from a Caesium 2000 source (experiment under analysis) and in the form of 131-I in a dose of 10μCi/rat, given by intraperitoneal injection. Goitrogen treatment was by administration of Sodium Perchlorate, 1g/100ml in the drinking water. Preliminary experiments established that perchlorate at this dosage was effective in maintaining a virtually complete block of iodide uptake by the thyroid gland. Further studies have established a reliable method of assessing numbers of tumours/gland by step sectioning at 100μ intervals and of measuring diameters of tumours. In this study a total of 157 rats were used; the first experiment assessed the number of tumours induced by goitrogen alone in comparison with goitrogen + 10uCi 131-I, the second experiment established the rate of appearance of tumours with time after radiation, and the third the effect of withdrawal of goitrogen on the persistence of tumours. Finally, and not reported in detail here, it was shown that temporary interruption of goitrogen treatment had no significant effect on tumour numbers. All sections were studied and lesions classified as either follicular hyperplasia or follicular tumour.

2. Results

(a) Comparison of tumours induced by goitrogen alone with those induced by μ goitrogen with radiation. After 282 days of goitrogen treatment the mean tumour number/rat lobe was 0.3 ± 0.6, while when 10μCi of 131-I was given the mean number rose to 4.6 ± 3.2 tumours/lobe. The mean tumour diameter, however, showed no significant difference between the two groups (683 vs 615μ).

(b) Rate of appearance of lesions with time after radiation
Animals killed at 0 days showed no tumours, those killed at 47 days showed 0.1 tumours/lobe, at 120 days 1.3 tumours/lobe (± 1.7), at 200 days 1.9 tumours/lobe (± 1.4) and at 282 days 4.6 tumours/lobe (± 3.2). This smooth progression was significant and a similar increase occurred in the number of follicular hyperplasias. The mean tumour diameter rose from 300u at 47 days to 568 μ at 120 days and then stabilised at 600-700μ for the remainder of the experiment.

(c) Effect of withdrawal of TSH stimulation on persistence of tumours
All animals were killed at the same age (282 days after start of goitrogen treatment). When goitrogen was withdrawn from animals after 47 days' treatment, no lesions were found; withdrawal after 120 days left 0.1 tumours/lobe, and after 200 days 0.7 tumours/lobe. The mean diameter of the remaining tumours in the last group was slightly greater (714 μ) than that seen in the animals remaining on goitrogen to the end of the experiment.

(d) Effect of brief interruption of goitrogen
This experiment was essentially negative - both for tumour size and number.

3. Discussion

This series of experiments shows that we have established a reliable quantitative assessment of tumour size and number for radiation thyroid carcinogenesis in rats. The number of thyroid adenomas induced increased smoothly with time after radiation, in the presence of continued goitrogen treatment; the size of the tumours increased rapidly and then plateaued as the increasing size of existing tumours was balanced by the generation of new small lesions. Goitrogen withdrawal at an early stage led to disappearance of all lesions; at later stages to disappearance of 90%, while after 200 days of goitrogen treatment about 40% of adenomas persisted. Detailed analysis of the size of the tumours suggested that the adenomas could be divided into two groups, with one group consisting largely of smaller lesions which showed their TSH dependence by disappearing after withdrawal of stimulus, while the second group consisted of larger lesions which showed at least partial lack of TSH dependence by persisting after withdrawal of the stimulus. These results are consistent with the clonal progression of thyroid adenomas; with the early lesions maintaining complete TSH dependence, and the later lesions losing TSH dependence. They are relevant to the role of elevated TSH in human radiation thyroid carcinogenesis and the need to inhibit this elevation for prevention of tumour formation.

IV. Objectives for the next reporting period:

To analyse in detail the results which have been obtained from both the studies using external radiation and those using I-131. To investigate the effect of prior thyroid growth on radiation carcinogenesis of the thyroid.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

None

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-212-NL

TNO Institute
for Experimental Gerontology
Juliana van Stolberglaan 148
Postbus 297
NL - 2501 BD 's-Gravenhage

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Zurcher
Instituut voor
Experimentele Gerontologie
Lange Kleiweg, 151, Postbus 5815
NL - 2280 HV Rijswijk

Telephone number: 015-13.69.40

Title of the research contract:

Flow cytometric analysis, computer aided morphometry and histopathology of radiation-induced rat mammary neoplasms as parameters for their biological behaviour.

List of projects:

1. Flow cytometric analysis, computer aided morphometry and histopathology of radiation-induced rat mammary neoplasms as parameters for their biological behaviour.

Title of the project no.:

Flow cytometric analysis, computer aided morphometry and histopathology of radiation-induced rat mammary neoplasms as parameters for their biological behaviour.

Head(s) of project:

Dr. C. Zurcher
Institute for Experimental Gerontology
Lange Kleiweg 151, P.O. Box 5815
NL - 2280 HV Rijswijk

Scientific staff:

Dr. C. Zurcher
Ir. M.J.J. Gijbels
Dr. J.J. Broerse
Dr. J.M.W. Visser

I. Objectives of the project:

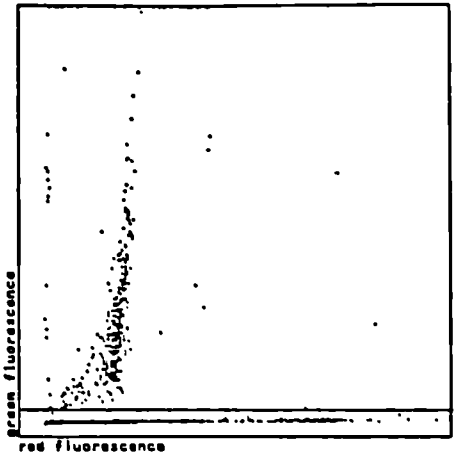
Assessment of various parameters of malignancy for spontaneous and radiation-induced rat mammary tumours, i.e. DNA flow cytometry, computer aided morphometry and histopathology.
Flow cytometry will be performed on single nuclei suspensions from paraffin embedded rat mammary tumour material. The histograms produced will be used to calculate the index of DNA aneuploidy and, if possible, the percentage of cells in S phase.
Morphometric analysis of the same selected mammary tumours will be performed on histological slides and on cytospin preparations of suspensions of nuclei used for DNA flow cytometry.
Flowcytometric and morphometric data will be correlated with histological signs of malignancy such as cellular pleomorphism, mitotic rate, invasiveness and metastasizing capacity.

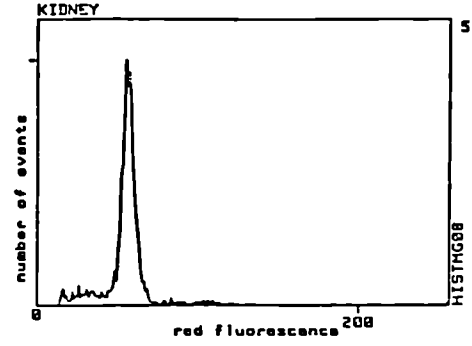
II. Objectives for the reporting period:

1. To investigate whether double staining of renal tissue nuclei, used as an internal standard, can be applied to recognize aneuploid populations and to distinguish periploid populations from artifacts inherent to formalin and embedding procedures.
2. To apply the standardized methods for DNA flowcytometry on formalin fixed archival tissues to the series of selected benign and malignant rat mammary tumours for estimation of DNA index and percentage cells in S phase.
3. To perform a morphometric analysis of the nuclei (area, ellipsoidity, regularity) of these rat mammary tumours on cytospin preparations and on histological slides selecting the most atypical or clearly malignant areas.

III. Progress achieved:

To provide an internal standard for normal diploid cells, nuclei of renal tissue of the same mammary tumour bearing rat were isolated and labeled with Fluoresceine Isothiocyanate (FITC). These nuclei were mixed with those isolated from the mammary tumour of the same rat and both types of nuclei were subsequently labeled with Propidium Iodide (PI). By measuring red (PI) and green (FITC) fluorescence a scatter plot resulted, which by windowing was used to provide two histograms: one representing renal nuclei (green + red) and one representing tumour nuclei (red) (fig. 1a,b,c). From these histograms the peak channel number of the first major peak of the tumour cell suspension was derived and divided by the peak channel number of normal renal nuclei.





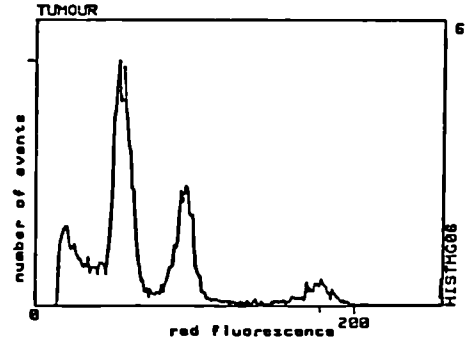


Fig. 1a: Scatter plot. Horizontal threshold distinguishes double stained renal nuclei (above) from tumour cells (below)
1b: histogram of part of scatter diagram above threshold.
1c: histogram of part of scatter diagram below threshold.

This resulted in the DNA Index (DI). By comparing peak channel numbers of PI only, and PI + FITC stained nuclei of the same renal tissue sample it appeared that in the presence of FITC a higher red fluorescence was observed. This factor could vary from 1.00 to 1.14. The FITC contributes somewhat to the red fluorescence. The amount of the contribution may vary with dye concentrations and filter choices. Therefore, for each run this factor was determined using the same standard parafinised renal tissue. DI indexes were corrected accordingly. Results obtained in tumour samples were arbitrarily considered as abnormal if exceeding the following values: DI 0.9-1.10; S phase: 1%; G2M phase: 7%. The method for preparation of nuclei was as follows: The renal nuclei were stained overnight with FITC (3µg/ml in 0.5 M sodiumbicarbonate pH 8.0). The nuclei were subsequently rinsed one time with aquadest to remove excess stain. Ten minutes before flowcytometry 0.5 ml of FITC labeled renal nuclei was mixed with 0.5 ml of tumour nuclei and the ensuing suspension was labeled with PI in a final concentration of 4 µg/ml.

Cellular DNA content was measured in at least 10.000 cells on a modified fluorescence activated cell sorter, RELACS-3 (3-laser Rijswijk Experimental Light Activated Cell Sorter) using an argon laser operating at 488 nm (0.5 W). Forward light scatter and time-of-flight measurements were conducted to elimimate aggregates and debris from the analysis. Simultaneously green and red fluorescence were measured. Data were collected in list mode and analysed as described above. As already mentioned DI was measured by the ratio peak channel of the main tumour peak/peak channel of renal nuclei. The locations of other peaks was related to that of the main tumour peak. Percentage S phase was estimated as the percentage of nuclei not present in the Gaussian distributions.

Abnormal DNA histograms were characterized by: abnormal location of the first peak (aneuploidy), additional increased second or third peaks, increased percentage of nuclei under S or G2M phase of first or additional peaks. Tumour and renal

tissue samples of 66 rats were examined. The histological diagnoses of the rat tumours ranged from benign (tubular adenoma, papillary cystadenoma, fibroadenoma) to noninvasive and invasive tubulopapilary and cribriform-comedo carcinomas and a few metastasizing carcinomas.

For those cases where an abnormal histogram was obtained the results are summarized in the table.

Abnormal DNA histograms were seen in 10 out of 27 benign mammary tumours, in 14 of 18 noninvasive malignant tumours, in 9 of 16 of the invasive malignant tumours and in 5 out 5 metastasizing carcinomas. Abnormal low (n=13) or high (n=2) DI values were observed in all tumour types in about the same frequency (range 19-26%). Therefore in the majority of histologically malignant rat mammary tumours the nuclei of the main tumour peak had a normal diploid DNA content. A high proportion of nuclei under the second peak and an increased percentage of S phase cells occurred more often in malignant tumours than in benign mammary tumours, with the highest figures obtained in the metastasizing tumours. In 23 of the 66 cases an increased second peak was observed. For the 4 main categories the frequencies were as follows: benign: 3/27; malignant, noninvasive: 10/18; malignant, invasive: 6/16 and metastasizing: 4/5. Furthermore, while in most cases the location of the second peak fitted the location of the G2M phase, in some of the malignant tumours the location of the second peak was somewhere between that of the first and second peak.

In conclusion: while abnormal DNA histograms increased in frequency with increasing signs of histological malignancy, although values for invasive tumours did not differ from those of noninvasive tumours, an appreciable proportion of histological malignant mammary tumours were unrecognizable as malignant by DNA flow cytometry alone. The same holds true for measurement of the mitotic index (data not shown). Morphometric studies of cytospin preparations were not feasible due to rapid detorioration of nuclear morphology when kept in suspension. Morphometry of histological sections is in progress.

Table

Rat Mammary Tumours with Abnormal DNA Histograms

Benign	DI	S_1 (%)	peak 2 (%)	peak 3 (%)	S_2 (%)
tubular ad.	0.84				
n = 8	0.93 (3.9)	1.81 (6.9)			
	1.02 (3.4)	2.03 (11.0)			
papillary	0.88				
cystadenoma	1.19				
n = 9	1.12	1.69 (14.0)			
fibroadenoma	0.95 (4.1)	1.84 (8.1)			
n= 10	0.79				
	0.83				
	0.77				
Malignant, noninv.					
tubulopapill.	0.86				
n = 9	0.95 (4.1)	1.80 (27.0)			
	1.01 (1.8)	1.73 (29.5)	3.25	(8.6)	(2.4)
	0.95	1.32 (7.0)			
	0.89				
	0.95	1.46 (18.9)			
cribriform-	1.08 (3.2)	1.77 (11.2)	2.80	(2.8)	(1.6)
comedo	0.98	1.66 (18.9)	2.06	(4.1)	
n = 9	1.04 (3.7)	1.88 (9.3)	3.33	(4.2)	(0.9)
	0.88				
	1.01	1.45 (12.6)			
	0.89 (3.0)	1.85 (42.4)	2.19	(2.9)	
	1.00	1.92 (9.7)			
	1.08 (1.0)	1.77 (12.0)			
Malignant, inv.					
tubulo	0.91 (2.6)	1.91 (8.5)			
papill.	0.86 (6.2)	1.97 (8.0)	2.88 (6.0)		(2.1)
n = 7	0.82				
	1.03 (2.1)	1.90 (8.1)			
	0.76				
cribriform	1.10 (2.5)	1.89 (13.0)			
comedo	0.98 (2.5)	1.72 (46.9)	1.88		
n = 9	0.97 (2.3)	1.95 (6.9)			
	0.93 (1.9)	1.71 (10.3)			
Metastasizing					
n = 5	0.87				
	1.03 (6.2)	1.91 (42.0)			
	0.91 (5.4)	1.96 (32.4)			
	0.99 (5.1)	1.88 (19.7)			
	1.03 (5.8)	1.94 (18.6)			

IV. Objectives for the next reporting period:

Morphometric analysis of nuclear charateristics in histological sections of a selected number of benign and malignant rat mammary tumours.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. J.J. Broerse
Dr. J.M.W. Visser
TNO Radiobiological Institute, P.O. Box 5815
2280 HV Rijswijk, The Netherlands.

VI. Publications:

None in this period of the contract.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-D-099-D

EULEP
Unité de Radiobiol. & Radioprot.
Université Catholique de Louvain
Avenue Hippocrate 54
B - 1200 Bruxelles

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.R. Maisin
UCL - Faculté de Médecine
Unité de Radiobiol.& Radioprot.
Avenue Hippocrate, 54
B - 1200 Bruxelles

Telephone number: 56-911661

Title of the research contract:

Late somatic effects of ionizing radiation on the mammalian organism.

List of projects:

1. Promotion of cooperation in the fields of late somatic effects, such as non-stochastic damage, carcinogenesis, effects of incorporated radionuclides and consequences of in utero exposure.

Title of the project no.: 1

Promotion of co-operation in the fields of late somatic effects, such as non-stochastic damage, carcinogenesis, effects of incorporated radionuclides and consequences of in utero exposure.

Head(s) of project:

Dr. J.R. Maisin
UCL-Faculté de Médecine
Unité de Radiobiol. & Radioprot.
Avenue Hippocrate 54
B-1200 Bruxelles

Scientific staff:

I. Objectives of the project:

The objective of EULEP is to plan, promote, execute and analyse co-operative research relevant to the understanding of the biological effects of exposure of living organisms to ionizing radiation.

II. Objectives for the reporting period:

1. To co-ordinate research activities on specific aspects of the late effects of ionizing radiation by means of problem-oriented task groups;
2. To maintain, develop and standardise relevant methodologies, to introduce new techniques, and to initiate appropriate training activities, by means of four committees:
   - Committee of External Radiation Dosimetry and Techniques
   - Committee of Internal Radiation Dosimetry and Techniques
   - Committee of Pathology
   - Committee of Cell and Molecular Biology;
3. To plan regular symposia on aspects of late effects studies.

III. Progress achieved:

Committee of External Radiation Dosimetry and Techniques

Appreciable progress has been made in the preparation for the sixth EULEP X-ray dosimetry intercomparison. At the RIVM, Bilthoven measurement and evaluation procedures have been completed, including testing of another type of TLD reader and its calibration. Recent information on W/e values and perturbation correction factors is included in the analysis. The new procedures have been tested in joint experiments between RIVM and RBI-TNO, Rijswijk. Mouse phantoms filled with thermoluminescent dosimeters (TLD) will be mailed to the participating EULEP laboratories during 1989.

High dose Total Body Irradiation (TBI) in combination with intensive chemotherapy followed by bone marrow transplantation (BMT) has shown to be of increasing benefit for the treatment of acute leukaemia and some disseminated diseases. Under the auspices of the EULEP, the European Society for Therapeutic Radiology and Oncology (ESTRO) and the European Bone Marrow Transplant Group (EBMT), an international meeting on Physical, Biological and Clinical Aspects of Total Body Irradiation was held at the Hague on 7-9 September 1988. The following aspects were covered: techniques and dosimetry for TBI, clinical basis for TBI fractionation, immunohaematological aspects of TBI and late effects after bone marrow transplantation. Clinical information on more than 2200 patients from 52 European centres has been collected. However, the different clinics used a variety of photon beams, fractionation schedules and dose rates. It will be important to investigate the influence of these physical factors in the occurrence of early and late complications.

Committee of Internal Radiation Dosimetry and Techniques

The committee has continued to give support to the six task groups which fall into its sphere of activity. Much effort has been given to the standardisation and intercomparison of experimental methods. In this respect the

work of the Task Group on Inter-species Comparison of Lung Clearance has been conspicuous; all the collaborating laboratories have standardised and compared their experimental methods for the holding culture of alveolar macrophages and for measurements of intra-macrophage pH.

The development of the simple computer program for the calculation of the absorbed radiation dose to 21 tissues, plus the absorbed dose equivalent, following inhalation or ingestion of any one of the 50 most important radionuclides has been completed. Copies of the program have been distributed to various member laboratories. The program - DOSELIB - is suitable for any IBM-compatible personal computer and copies are available on disc.

Contacts with the EURADOS Committee on Assessment of Internal Dose have been established and, in view of the overlapping interests of the two committees, it has been recommended that each committee should send one representative to the other.

Committee of Pathology

A workshop on neoplastic and non-neoplastic lesions of haemopoietic tissues was organised by the committee of pathology on November 4 and 5, 1988 in Munich.

Six non-EULEP experts in the field were invited as guest speakers elaborating on the application of immuno-histochemical and molecular biological methods in research on malignant lymphomas in mice and man. Comparability with respect to histogenetic classification and aspects of biological behaviour such as progression to more malignant forms was evident in several studies. The meeting was attended by 25 scientists from 7 countries.

Other activities of the committee included the production of a new fascicle of the EULEP Pathology Atlas on lesions of the genital system, and the evaluation of a limited number of consultation cases.

For 1989 a seminar is planned on myeloid leukaemias in man and experimental animals.

Committee of Cell and Molecular Biology

The Committee has maintained regular contact with the task groups on Molecular Biology of Osteosarcoma and Molecular Biology of Lymphoma, and promoted a joint meeting of these groups in Bordeaux on September 29-30, 1988. Scientific results were presented from seven participating laboratories: Liège, Mol, Bordeaux, MRC Harwell, Neuherberg, Aarhus and Antwerp. The subjects discussed are included in (a) below.

Efforts are underway to further integrate the leukaemia studies into a common approach, based on the myeloid leukaemia model in the CBA mouse. With regret it has been accepted that the INSERM laboratory at Bordeaux will not in future be able to participate in the EULEP programme.

Training activities continue to be promoted by the committee, notably by exchange visits of scientists between laboratories to learn new techniques. This policy will be maintained in 1989, together with further joint meetings with the two task groups.

TASK GROUPS: Some of the more significant items of progress achieved by the co-ordinated work of the task groups were as follows:

(a) Molecular approach to the study of radiation-induced osteosarcoma and lymphoma

The murine osteosarcoma model has continued to provide useful insights into the mechanism of carcinogenesis at the molecular level. The participation of retroviruses remains a major focus of interest, together with the role of certain oncogenes. There is also at present a search for bone-specific differentiation genes.

The molecular studies on radiation-induced thymic lymphomas in rodents have progressed along broadly similar lines, and since the laboratories involved are not the same as those studying the osteosarcoma model, it has been important for these two task groups to maintain close liaison. The participation of retroviruses in thymic lymphoma induction has

continued to be of central interest. Work has been undertaken on the role of the presumed oncogene Mlvi-1, as well as on the phenotypes of preleukaemia cells and on the role of cytokines.

Attention is increasingly turning towards the myeloid leukaemia model in the CBA mouse. Recent studies have concentrated on specific chromosomal lesions commonly found in leukaemic cells, and on the possible activation of genes resulting from these lesions which may be concerned with leukaemogenesis itself.

(b) Cellular basis of late vascular changes in the areas at risk in the irradiated brain

Progress has been made in solving the problems to delineate and evaluate areas at risk, to evaluate the risk, and to evaluate factors that modify the expression of late vascular effects. The approach combines a number of morphological and physiological factors, with sampling times over one to two years.

Further analysis in this co-operative, multicentre study has revealed that in the parenchyma of the brain the white matter represents the most sensitive structure, and in the white matter the fimbria hippocampi. Extensive correlation studies have shown that the level of the dose, as well as the extent of time after irradiation, both strongly influence the eventual development of late tissue damage. The earliest phenomenon encountered was that of a blood vessel-related phenomenon, i.e., a "tissue injury unit", consisting of enlargement of endothelial cells, plus enlargement of astrocytes, blood vessel dilatation, and blood vessel wall thickening. Also when examined with E.M., primordial telangiectatic lesions selectively developing in fimbria hippocampi were found. Appearance of this entity preceded the development of signs of damage to the nervous system itself, i.e., demyelination. Moreover, the radiosensitivity as well as the radioresponsiveness of this lesion exceeded that of other components of the nervous tissue investigated so far. Circulation physiology indicated an absence of leakage of the blood brain barrier, and a decrease in nutritional blood flow.

(c) Effects of irradiation on pre-implantation mouse embryos

The radiosensitivity of pre-implantation mouse embryos was studied regarding the following aspects: induction of developmental abnormalities, induction of chromosome aberrations and micronuclei, changes of protein synthesis and phosphorylation. Developmental abnormalities were mainly induced during the first hours after conception (one-cell stage). The radiosensitivity changed within hours. Similar results were obtained for the induction of chromosome aberrations. The development of chromosomal damage was followed through several cell cycles after irradiation. These studies were performed with X-rays and neutrons in Essen. In Mol and in Essen the phosphorylation and synthesis of proteins was studied in these developmental stages. These processes are important for the regulation of the development. Several proteins could be identified which are responsible for the processes. The influence of caffeine, which has a radiosensitising activity, was also studied.

(d) Interspecies comparison of lung clearance

The first interspecies comparison of particle clearance from the lung, using relatively soluble porous cobalt oxide particles, has been followed by a second smaller study using less soluble 'solid' cobalt oxide particles. A further follow-up study is comparing the extent of retention in the respiratory tract of soluble (ionic) cobalt, which was suggested from the first study as part of the basis for the different patterns of translocation to blood among the various species.

There is now a major effort to study interspecies differences in the rate at which particles dissolve in the lung by comparing alveolar macrophages studied in vitro. Here the pH in the phagolysosomes may be an important factor, and the method for measuring it by fluorescence spectrometry, using fluorescein bound to silica particles, has been introduced into more participating laboratories by means of exchange visits between them. Efforts are being made to improve the viability of short-term cultures of macrophages

when these are transported from one institute to another. A method for immobilising the cells in microbeads of alginate gel appears promising for some of these studies, and will be further evaluated.

Other work by members of the group has been on nuclear aberrations in mouse macrophages following exposure to $^{239}PuO_2$.

(e) Treatment after incorporation of actinides

Studies on the efficacy of the compound LICAM(C) for the decorporation of plutonium and americium have been completed.

Future efforts to identify compounds that remove actinides from the body better than DTPA will require the provision of adequate amounts of new substances. Small quantities of DFO-HOPO and 3,4,3-LIHOPO have been made available from Berkeley, California and the latter was found to remove ^{239}Pu-TBP from rat serum in vitro more effectively than LICAM(C) or DTPA. Means of acquiring larger amounts of LIHOPO for animal experiments are being explored.

In the meantime other compounds have been tested as possible decorporating agents, without positive results as yet.

Past experience has demonstrated the value of collaborative effort between EULEP laboratories in this area, and opportunities are awaited to utilise the joint facilities of the task group when suitable materials for testing become available.

(f) Stem cell studies after contamination with actinides

Studies have continued on the effects of alpha-emitting radionuclides on stem cells and on stromal cells in the bone marrow, studied both in vitro and in vivo. In vitro studies are based on long-term bone marrow cultures.

The collaborative in vivo studies have arisen from previous work demonstrating the non-uniform distribution across the marrow space of stem cells at different stages of maturation. The effects of ^{239}Pu and ^{224}Ra have been compared both on axial marrow, which includes the most primitive of the

progenitor cells, and on marginal marrow, containing more mature cells and receiving higher doses of radiation from the bone. Recovery of stem cells (CFU-S) took place within 3 months of Pu administration, but there was a long-term loss in stem cell quality. Recovery was better after ^{224}Ra treatment, but took longer than the ^{224}Ra decay time, probably because of damage to the micro-environment.

Within 2 hours of ^{224}Ra administration a marked shift has been found in stem cells from the marginal region to the axial part of the marrow. The significance of this migration remains to be clarified.

Detailed studies have also been made in joint work on CFU-S stem cells in fetal liver and in neonatal spleen and bone marrow following ^{239}Pu administration to the mother at 4 and 13 days gestation. A marked reduction was seen in the numbers of CFU-S in the marginal bone marrow.

(g) Metabolism, dosimetry and effects of bone-seeking radionuclides

Long-term studies have continued in several participating laboratories, among which is an extensive investigation into the comparative distribution and local dosimetry of ^{233}U, ^{239}Pu and ^{241}Am in the mouse skeleton. Another long-term collaborative project concerns Pu and Am distribution in the bones of the baboon.

The carcinogenic effect of low doses of ^{224}Ra in the NMRI mouse has been shown to depend on dose protraction. A lymphoma incidence of 13.5% was seen with twice-weekly injections resulting in a mean skeletal dose of ca. 1 mGy/day, amounting finally to 0.15 Gy. The lymphomas were not seen if the same amount of ^{224}Ra was given in a single injection.

A large-scale study is in progress on the effects of low doses of ^{224}Ra in the CBA mouse. This is yielding significant numbers of cases of myeloid leukaemia, so far well in excess of the incidence of osteosarcoma.

(h) Effects of radiation on the development of the CNS

Investigations co-ordinated by the task group cover

different aspects of the initiation of damage to the CNS, chains of reactions interfering with subsequent developmental processes, and different manifestations of final damage. Irradiation with mice, rats and rabbits have been at the stages of advanced organogenesis, early fetogenesis and the early postnatal stage. Within the different studies the doses administered ranged from high levels giving marked effects down to the lowest dose leading to any detectable effect.

Biochemical studies on postnatal levels of serotonin receptors, vascular functions and myelin lipids in rat brain, after 0.95 Gy X-rays 12 or 15 days p.c., suggested that late changes involved mainly the neurones.

Areas of the brain in which cell proliferation persists postnatally have been related to radiosensitivity at that stage in the gyrus dentatus, in the hippocampal formation, in the external granular layer of the cerebellum, and in the olfactory bulb. In the rat the cingular cortex is especially associated with low-dose morphological responses.

The manifestation of neuronal damage in the cortex in terms of disorganisation of nerve processes, disturbed proliferation and migration of neurones, branching defects and so forth has been extensively studied. The dose response curves obtained indicate threshold values for the induction of neuronal structure defects in the vicinity of 0.1 Gy.

A joint project was established on lectin binding studies. Effects were seen in the ependymal area with doses as low as 0.125 Gy.

(i) Effects of residual radiation injury in dermal and subcutaneous vascular/connective tissues on subsequent skin exposure

Experiments have continued on problems associated with the standardisation of methodologies between the two participating institutes. Their previous work utilised different strains of pig and different anaesthetics during irradiation.

Using the same anaesthetic [illegible]peron/etomidat - the large white pigs showed a higher [illegible] moist desquamat[illegible]

than the Gottinger minipigs, the difference being even greater than when the large white pigs were anaesthetised with halothane/N_2O/O_2. It appears that azaperon/etomidat induced hypoxia in the skin of the large white pigs. This also led to variations in ED_{50} between different skin fields.

Further experiments are planned to take into account the different radiation sources used (beta and gamma) in the two laboratories, as well as the anaesthetic agents.

(j) Cell biology of lyphomas

Until now the task group has been concerned with collaborative studies on thymic lymphoma, using mainly two indirect models for investigating cellular factors, i.e. thymic graft to thymectomised irradiated animals; and transfer of preleukaemic cells (and/or factors) to irradiated recipients.

All the participating groups are actively looking for the role of lymphokines after irradiation, during leukaemogenesis in vivo and during the long period preceding the establishment of lymphoma lines in tissue culture. The involvement of Il-6 has been suggested from some of the work. In situ hybridisation studies are in progress.

There is also some interest in receptors for neurotransmitters such as VIP, which can interact with lymphocyte functions.

In future years it is expected that there will be increasing emphasis on myeloid leukaemia and also on human pathology.

(k) Retention and absorption of ingested radionuclides and irradiation of the gastrointestinal tract

Co-ordinated studies on the intestinal retention of plutonium in neonates have demonstrated clear species differences. For example, 5 days after administration of ^{238}Pu citrate to 6 day old animals, the small intestine retained ca 20% of the amount given to rats and ca 0.02% in guinea pigs. The location of Pu also differed between these species, being mainly epithelial in rats but subepithelial in

the guinea pig; in neither however was the activity located near to the crypt cells. Nor was there significant retention in lymphoid tissue.

Limited studies with baboons showed similar levels of retention and a similar distribution pattern to those in the guinea pig, suggesting that the latter may be an appropriate model for man. In that case, plutonium retention in the human neonatal intestine is likely to be low.

Completion of the interspecies comparison will require ultrastructural investigations of intestinal enterocytes.

Future work may extend to actinide absorption measurements in human volunteers, in addition to animal studies on age-related changes in absorption and related topics.

(l) Fetal dosimetry and effects of incorporated radionuclides

Much of the work of the task group has involved studies of the transfer of actinides to the fetus and their effects. Two laboratories are developing dosimetric models for the fetus. Two others have examined the effects of external radiation and internally incorporated radionuclides on the developing brain (see (h) above). Others are concerned with the transfer of radionuclides to both the fetus and the neonate, and there is increasing interest in assessing the consequences of the intake of naturally occurring radionuclides, e.g. the transfer of ^{210}Po and ^{210}Pb to the fetus.

Extrapolation of data from rodents to man will require further consideration of embryological development of fetal membranes and tissues in rodents.

During 1988 the task group organised one workshop at Reisensburg on March 8, and another at Harwell on November 8-9 jointly with the task groups on stem cells and on bone-seeking radionuclides.

IV. Objectives for the next reporting period:

The programmes of the committees and task groups will be continued as outlined above. Greater emphasis will be placed on the development of various forms of training activities. The sixth X-ray dosimetry intercomparison will take place in 1989. A symposium is planned on "skin-related problems in radiation accidents and radiological protection".

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

EULEP Pathology Atlas: Lesions of the Genital System.
EULEP Newsletters 47, 48, 49 and 50.

III E

GENETISCHE WIRKUNGEN IONISIERENDER STRAHLEN

GENETIC EFFECTS OF IONIZING RADIATION

EFFETS GENETIQUES DES RAYONNEMENTS IONISANTS

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-148-NL

Organisation for Health Research
TNO
Juliana van Stolberglaan, 148
NL - 2595 CL Den Haag

Head(s) of research team(s) [name(s) and address(es)]:

Dr. R.A. Baan
Medical Biological Laboratory
TNO
P.O. Box 45
NL - 2280 AA Rijswijk

Telephone number: 15-13.87.77

Title of the research contract:

The genetic and biochemical basis of radiation sensitivity in cultured human and other mammalian cells.

List of projects:

1. Biochemical analysis of DNA repair functions in mammalian cells.

Title of the project no.: 1

Biochemical analysis of DNA repair functions in mammalian cells

Head(s) of project: Dr.G.P. van der Schans and Dr. F. Berends.

Scientific staff: Dr. G.P. van der Schans and Drs. L. Roza

I. Objectives of the project:

This project aims at the identification of various DNA lesions in irradiated mammalian cells and the elucidation of their repair. Special attention will be given to the development of methods for the detection of a variety of lesions in DNA of irradiated mammalian cells, and on the study of their repair. The agents which will be used in these studies for the induction of lesions are ionizing radiation and ultraviolet light of different wavelengths.

II. Objectives for the reporting period:

Our attention was focussed on 2 approaches for the detection of various radiation-induced lesions:
(i) immunochemical methods: Production of antibodies against radiation-induced lesions (thymine glycols, local single-strandedness) and development of immunochemical assays based on the antisera obtained
(ii) biochemical methods: Development of an alkaline elution method for the senstive detection of single-strand breaks, with fluorometric quantitation of DNA, meant to be applicable after in vitro as well as in vivo irradiation.

Further, repair of UV- and ionizing radiation-induced lesions was studied.

III. Progress achieved:

Methodology:

For the immunochemical detection of radiation damage, specific antisera are needed. Small pieces of modified DNA were synthesized, in which the modification corresponded to one of the radiation-induced damages; against these "DNA-damages" antibodies are being raised. One such "DNA-damage" synthesized was the thymine-dimer in the tetranucleotide GpTpTpG. This lesion, the main damage induced by ultraviolet light (UV), is also important as a product of ionizing radiation. For another lesion induced by ionizing radiation, hydroxymethyldeoxyuridine (HMdU, an oxydation product of thymine), commercially available hydroxymethyluridine (HMU) was chosen as the antigen. For thymine glycols, induced by ionizing radiation, OsO_4-treated poly(dT) was prepared. All these products, coupled to a carrier protein, were used for immunizations.

Polyclonal antisera

The polyclonal rabbit-antiserum applied during this reporting period, was raised against poly(dT) treated with OsO_4 to induce thymineglycols, as described in the foregoing progress report.

Monoclonal antibodies

These antibodies were obtained after immunizations of mice with (i) OsO_4-treated poly(dT), coupled to methylated bovine serum albumin and (ii) GpTpTpG containing the thymine dimer, coupled to chicken-γ-globulin as carrier. From spleen cells of these mice, after fusion with muose myeloma cells, a large number of hybridomas were obtained which were selected for antigen-specificity. As a by-product from fusions of mouse myeloma cells with spleen cells isolated from a mouse immunized with DNA treated with benz(a)pyrene-diolepoxide, a monoclonal antibody (D1B) directed against single-stranded DNA was obtained.

Immunochemical detection methods

Immunochemical detection was carried out in 3 different ways:
(i) Direct ELISA, in which the lesion-containing DNA was coated to the wall of the wells in a 96-well plate and the amount of antibody-binding was detected by binding of a second antibody, directed against the first and conjugated to an enzyme which converts a substrate into a light-absorbing or fluorescing product. (ii) "Competitive" ELISA: lesion-containing DNA was first mixed with a fixed amount of antibodies; the excess of antibodies was backtitrated as described under (i). (iii) Lesions in DNA in cells fixed on glass slides were detected by binding of antibodies; quantitation was by means of binding of a second antibody carrying a fluorescing substituent.

Detection of single-strand breaks (SSB) by alkaline elution

Sensitive detection of SSB occurs via alkaline elution of DNA through membrane filters and fluorometric quantitation of the eluted DNA. This method permits the detection of radiation-induced SSB as well as lesions recognized by damage-specific endonucleases (e.g. "UV-endo"). These "UV-endo-sites" were also assayed via alkaline-sucrosegradient centrifugation.

Immunochemical detection of radiation-induced single-strandedness

This method is based on the binding of anti-single-stranded-DNA monoclonal antibody to single-stranded DNA. Local single-strandedness is induced by ionizing radiation at each single-strand break induced and at

other damages leading to distortion of the double-helix. This limited single-strandedness can be amplified enormously by a controlled time-dependent partial unwinding of the cellular DNA under strictly defined, mildly alkaline conditions. After neutralization, immediately followed by sonication (to prevent restoration of the double-helix), the percentage of single-strandedness can be detected in a competitive ELISA (the 100% value is determined by performing sonication before neutralization). The percentage of single-strandedness is a measure for the amount of damage induced in the DNA. The method is rapid, does not require radioactive labelling of DNA, and is sufficiently sensitive to detect damage induced by 1 Gy of ionizing radiation.

RESULTS AND DISCUSSION

Ionizing radiation damage

In the development of sensitive methods for the detection of various radiation-induced lesions and their repair, two approaches are followed. (i) immunochemical detection of radiation damage; (ii) sensitive detection of DNA breaks. The studies aim at methods applicable to white blood cells and suitable for the monitoring of human populations.

(i): Immunization of rabbits with OsO_4-treated poly(dT) yielded IgG-antibodies, which react specifically with DNA treated with a low concentration of OsO_4. After chromatographic fractionation of the polyclonal antiserum some fractions could be isolated with which it was possible to detect thymine glycols in mammalian cells exposed to 20 Gy of ^{60}Co-γ-rays. The attempts to prepare more specific monoclonal antibodies against thymine glycol, which would lower the detection level to 1-10 Gy, failed so far.

The immunochemical assay of radiation-induced single-strandedness, amplified by mild alkaline treatment, allows detection of damage induced with 1 Gy of radiation, both in exposed human blood and in mice (in the white blood cells as well as the bone marrow cells). Not only the rapid repairable single-strand breaks can be detected by this method but also some other, more persistent damage, both in human white blood cells and murine white blood cells and bone marrow cells. This technique now has been simplified in such a way that much more samples can be analysed within a shorter time. Furthermore, attempts were initiated to isolate monoclonal antibodies against single-stranded DNA of the IgG type to replace the IgM-type antibodies presently applied. Since the accessibility of lesions for IgG is expected to be higher than for IgM, these antibodies would be particularly useful for the detection of damage at the single-cell level.

The method was applied in a collaborative study with Dr. A. Grootegoed (Biochemistry II, Erasmus University, Rotterdam) on the induction and repair of damage in germ cells in different stages of spermatogenesis of the Syrian goldhamster. In all stages an initially rapid removal followed by a slow repair of damage was detected. However, in the stage just before the conversion into spermatozoa, the so-mentioned elongated spermatids, no significant repair was observed during the first 60 min, both after *in vivo* and *in vitro* exposure.

(ii): With the alkaline elution method, DNA breaks after irradiation of both human blood and mice with ionizing radiation could be measured down to 1 Gy. In irradiated mice, the induction and repair of SSB could be studied not only in the DNA of the white blood cells, but also in the bone marrow cells (in the whole population of cells from a femur). Experiments are in progress for studying the induction and repair of SSB in gut cells after irradiation of mice.

Furthermore, with alkaline elution it was established that a radiation-sensitive mutant derived from V79 Chinese hamster cells (XR-V15B) has normal removal of SSB, whereas with neutral elution it could be shown that after 4 h of repair more than 50% of the double-strand breaks remained, in comparison to 3% in V79 cells.

Ultraviolet light (UV) induced damage

Irradiation of cultured human cells with UV leads to DNA damage, which may result in cell death or mutations. In our investigations on dimers vs. non-dimer UV-lesions, with human and rodent cells in vivo and in vitro irradiated with UV-C (254 nm), UV-B (280-320) or UV-A (320-380 nm), various phenomena were studied:

(i): Repair of pyrimidine dimers. Removal of UV-endonuclease susceptible sites was much less in Chinese hamster cells than in human cells. In a Chinese hamster cell mutant, which almost completely lacks repair of pyrimidine dimers, the removal of these lesions appeared to be restored to the level of the wild type cells after the introduction of the human ERCC1 gene. In another mutant of V79 Chinese hamster cells (V-B11), belonging to a new (seventh) complementation group, accumulation of incision breaks after UV-C irradiation during incubation in the presence of hydroxyurea and β-arabinofuranosylcytosine, at 2 h after UV exposure, was about 30% of that found in wild type cells. Furthermore, phenotypic heterogeneity within the first complementation group of UV-sensitive mutants of Chinese hamster cell lines could be demonstrated by using the same technique.

(ii): Photoreactivation of UV damage. This has been described for human cells irradiated with UV-C or UV-B. In the past we were unable to demonstrate photoreactivation in cultured human cells. As it appeared possible that cultured cells do not maintain all repair capabilities of the cells in vivo from which they originate, we wished to study photoreactivation in humans. This would require specific and extremely sensitive detection of dimers in small amounts of no-radioactive DNA. Immunochemical detection with specific antibodies appeared the method of choice.

Monoclonal antibodies from hybridomas obtained after fusion of spleen cells of mice, immunized with GpTpTpG containing the T-T dimer, also recognize thymine dimers in DNA. Immunochemical detection with the use of this antibody appeared to be sufficiently sensitive for our study on the induction and repair of UV-damage in human skin cells, both in vitro and in vivo, and on the occurrence of photoreactivation. Experiments with cultured human cells injected with yeast photoreactivating enzyme, indicated that photoreactivation of thymine dimers, if occurring, should be demonstrable with this assay. However, no photoreactivation could be observed with non-injected cells, which confirmed our earlier conclusion about the absence of this process in cultured human cells. Further results with microinjected cells showed that - surprisingly - illumination immediately after UV-irradiation does not reduce UDS, whereas photoreactivation at later moments (1 h) results in a substantial decrease of UDS during the subsequent period. These data suggest that the initially detected UDS is mainly due to types of damage other than thymine dimers, possibly (6-4)photoproducts, of which the removal by the incision-excision repair mechanism is much faster than that of pyrimidine dimers.

With the same antibodies, thymine dimers can be detected in situ, with immunofluorescence microscopy, in cryostat sections of UV-B-irradiated human skin (200 mJ/cm^2). Quantitation of the fluorescent signal is difficult since not all cells are in focus and because the fluorescence varies with the distance to the horny layer. Nevertheless, it appeared to be possible to obtain a dose-effect relation by averaging the fluorescence over all cells in the epidermal layer that are in focus. The results obtained compared well with the data resulting from measurements on cell suspensions prepared from irradiated skin. None of the persons exposed to UV-B, followed by a partly exposure to photoreactivating light, showed an enhanced removal of dimers in comparison to parts not exposed to this light. For one person, exposed to a fractionated dose of UV-B and subsequent illumination after each UV-B irradiation, an enhanced removal of pyrimidine dimers was observed. Two other persons did not show this phenomenon. This suggests that photoreactivation might be an inducible process, but only in certain individuals.

IV. Objectives for the next reporting period:

As in the foregoing reporting period, our attention will be focussed on the approaches mentioned before:
(i) Immunochemical and biochemical detection of radiation damage. The very promising method of detection of single-strandedness will be extented to the detection of base damage which inside the cell first is converted (enzymatically or chemically) into a single-strand break or an alkali-labile site. Beside the antibodies directed against the lesions already in study, antibodies will be raised against the (6-4)dithymine fotoproduct and its Dewar-isomer, induced by UV in DNA.
(ii) Further development of the alkaline elution method, particularly with respect to the detection of DNA-DNA and DNA-protein crosslinks induced by ionizing radiation.
(iii) Application of the developed methods for the study of repair mechanisms.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

This research concerns part of a collaborative programme of the MRC Cell Mutation Unit, Falmer, Brighton, Sussex, UK (Prof.Dr. B.A.Bridges) and the Dutch laboratories: Department of Genetics, Erasmus University, Rotterdam (Prof.Dr. D.Bootsma), Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden (Dr.A.A. van Zeeland), Department of Molecular Genetics, University of Leiden (Prof. Dr. P. van de Putte), Department of Medical Biochemistry, University of Leiden (Prof. Dr. L. van der Eb) and the Medical Biological Laboratory TNO, Rijswijk (Dr. R.A. Baan)

VI. Publications

Boerrigter, M.E.T.I., E. Mullaart, G.P. van der Schans and J. Vijg (1989) Quiescent human peripheral blood lymphocytes do not contain a sizable amount of preexsistent DNA single-strand breaks. Experimental Cell Res. (in press)

Mullaart, E., P.H.M. Lohman and J. Vijg (1988) Differences in pyrimidine dimer removal between rat skin cells in vitro and in vivo. J. Invest. Dermatology, 90, 346-349.

Mullaart, E., L. Roza, P.H.M. Lohman and J. Vijg (1988) The removal of UV-induced pyrimidine dimers from DNA of rat skin cells in vitro and in vivo in relation to aging. Mech. Aging Develop. (in press)

Jaspers, N.G.J., L.Roza, W. Vermeulen, A. Eker, R.D.F.M. Taalman, J.H.J. Hoeijmakers and D. Bootsma (1989) In vitro correction of cells from patients with mutagen hypersensitivity. Progress of Int. Congress on DNA repair, Rome (Italy) (in press).

Roza, L., R.A. Baan, J.C. van der Leun and L. Kligman (1989) UVA hazards in skin associated with the use of tanning equipment. J. Photobiol and Photochem. (ed. A.R. Young) (in press).

Roza, L., K.J.M. van der Wulp, S.J. MacFarlane, P.H.M. Lohman and R.A. Baan (1988). Detection of cyclobutane thymine dimers in DNA of human cells with monoclonal antibodies raised against a thymine dimer containing tetranucleotide. Photochem. Photobiol. 48, 627-633.

Schans, G.P. van der (1989) The induction and repair of double-strand breaks in mammalian cells as detected by neutral elution. Progress of Int. Congress on DNA repair. Rome (Italy) (in press).

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Vijg, J., L. Roza, E. Mullaart and F. Berends (1987) DNA repair in relation to skin aging. Giornale Italiano Di Dermatologia Chirurgica, 2, 300-311.

Zdzienicka, M.Z., N.G.J. Jaspers, G.P. van der Schans, A.T. Natarayan and J.W.I.M. Simons (1989) Ataxia-telangiectasia-like radioresistant DNA synthesis, chromosomal instability and normal DNA strand break repair. Cancer Res. (in press).

Zdzienicka, M.Z., G.P. van der Schans and J.W.I.M. Simons (1988) Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster cells. Mutation Res. 194, 165-170.

Zdzienicka, M.Z., G.P. van der Schans, A. Westerveld, A.A. van Zeeland and J.W.I.M. Simons (1988) Phenotypic heterogeneity within the first complementation group of UV-sensitive mutants of Chinese hamster cell lines. Mutation Res. 193, 31-41.

Zdzienicka, M.Z., Q. Tran, G.P. van der Schans and J.W.I.M. Simons (1988) Characterization of an X-ray-hypersensitive mutant of V79 Chinese hamster cells. Mutation Res. 194, 239-249.

Short communications, abstracts...

Roza, L., J.B.A. Bergen Henegouwen and R.A. Baan (1988) Detection of UV-induced DNA damages in UV-irradiated human skin. Proc. of the 29th Dutch Federation Meeting, Utrecht, Dutch Federation of Medical Scientific Societies (abstract)

Roza, L., C.J.M. van der Wulp, W. Vermeulen and R.A. Baan (1988) Relative quantification of UV-induced DNA lesions in single cells by immunofluorescence microscopy. J.Soc. Anal. Cytology, 1988 (suppl.2), 22 (abstract).

Schans, G.P. van der, A.A.W.M. van Loon, R.H. Groenendijk and R.A. Baan. (1988) Detection of DNA damage in cells exposed to ionizing radiation by use of anti-single-stranded-DNA monoclonal antibody. Book of abstracts, Joint Meeting on Experimental and Clinical Radiobiology of the Netherlands, Belgian and Swedish societies for Radiobiology, the British Association for Radiation Research and the Radiobiology Committee of the British Institute of Radiology, Noordwijkerhout, May 1988, The Netherlands (abstract).

Zdzienicka, M.Z., Q. Tran, G.P. van der Schans, N.G.J. Jaspers and J.W.I.M. Simons (1988) Chinese hamster V79 cell lines hypersensitive to ionizing radiation. Book of abstracts, Joint Meeting on Experimental and Clinical Radiobiology of the Netherlands, Belgian and Swedish societies for Radiobiology, the British Association for Radiation Research and the Radiobiology Committee of the British Institute of Radiology, Noordwijkerhout, May 1988, The Netherlands (abstract).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-E-204-I

Università degli Studi di Milano
via Festa del Perdono, 7
I - 20125 Milano

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M. Bianchi
Dip. Genet. & Biol. dei Microorg.
Università degli Studi di Milano
via Celoria, 26
I - 20133 Milano

Telephone number: 02-299291

Title of the research contract:

Development of biochemical and immunological assays for DNA recombination and repair

List of projects:

1. Purification and characterization of the eukaryotic analogs of recA protein.
2. Development of immunological reagents and analysis of the induction of the recA analogs following DNA damaging treatments.
3. Cloning of the genes encoding the analogs of recA protein in yeast and mammalian cells.
4. Development of a general method for the identification of proteins involved in DNA recombination and repair.

Title of the project no.:

1. Purification and characterization of the eukaryotic analogs of recA protein.
2. Development of immunological reagents and the analysis of the induction of the recA analogs following DNA damaging treatments.
3. Cloning of the genes encoding the analogs of recA protein in yeast and mammalian cells.

Head(s) of project:

Dr. Marco Bianchi
Dr. Giampiero Sironi

Scientific staff:

Dr. Marco Bianchi
Dr. Giovanna Lucchini
Dr. Lucia Panzeri

I. Objectives of the project:

The identification of DNA recombination proteins in yeast and mammalian cells. The development of suitable assays to determine their expression levels under various physiological conditions, and following irradiation or exposure to DNA damaging agents.

II. Objectives for the reporting period:

The development of a general assay procedure for proteins that are involved in DNA repair and recombination.

III. Progress achieved:

METHODOLOGY

In the previous reporting period, we discovered that polyclonal antibodies raised against purified recA protein are not a sufficiently reliable reagent for the identification of recA analogs in eukaryotes, despite being an excellent reagent when used for the identification of recA analogs in prokaryotic species. The protein that was strongly recognized by the anti-recA antibodies in the yeast *Saccharomyces cerevisiae* (and could be induced by UV treatment) turned out to be a subunit of ribonucleotide reductase; the determinant for recognition was localised to the four C-terminal amino acids of this polypeptide, which are identical to the corrisponding amino acids of recA. For this reason we have discontinued the use of the anti-recA antibodies and have tried to exploit, as an alternative means to identify the eukaryotic recA analogs, the binding properties of recA and recA-like proteins to specific DNA structures. Holloman and co-workers have shown that the protein rec1, a recA analog from the fungus Ustilago maydis, can bind with high affinity to Z-DNA, presumably because Z-DNA is an intermediate formed in the enzymatic reaction catalysed by rec1 (Kmiec et al., Cell 40, 139-145). We have investigated whether binding to Z-DNA is a general property of recombination proteins, and whether it can be used as an assay for their purification. We also checked whether binding to other specific recombination intermediates can be used as an assay.

RESULTS AND DISCUSSION

In collaboration with Dr. Alfred Nordheim (ZMBH, Heidelberg), we have investigated whether binding to Z-DNA is a property of rec1 protein alone or is a general property of recombinases, and therefore shared also by recA.

An alternating GC sequence, capable of forming Z-DNA under the effect of supercoiling, was cloned in pBR322. A fragment of approximately 400 bp, which contains the GC sequence, is cut from the plasmid, labeled with P32 and religated in vitro in the presence of variable amounts of ethidium bromide. The extraction of the ethium bromide after the ligation generates miniplasmids containing a limited, quantized number of superhelical turns; each topoisomer has a distinctive electrophoretic mobility and is easily distinguishable. Topoisomers with 0 or -1 superhelical turns do not contain Z-DNA, topoisomers with -2 or -3 superhelical turns contain Z-DNA.

When exposed to a monoclonal antibody which recognizes Z-DNA, topoisomers -2 and -3 form protein-DNA complexes with a different electrophoretic mobility, topoisomers 0 and -1 are unaffected. We tested whether recA formed complexes with topoisomers -2 and -3, but failed to detect any. This result is at variance with a presumed general ability of recombinases to interact with Z-DNA, and can be explained in two ways. One possibility is that Z-DNA binding is a peculiar property of rec1, not shared by recA. The other possibility is that rec1 and all other recombinases do not recognize Z-DNA per se, but some other structural feature present in the Z-DNA molecules that were used in previous studies, such as bromine substituents or small regions of denatured DNA. In any event, binding to Z-DNA does not look like a viable assay for eukaryotic recombinases.

We have also investigated whether recA can bind to triple-stranded DNA formed transiently during recombination. Reporter molecules containing triple-stranded DNA have been constructed as described by Francois et al. (Nucleic Acids Res. 16, 11431-1440). Preliminary results indicate that recA can bind to such structures, but further verification with control DNA molecules is needed.

IV. Objectives for the next reporting period:

To verify that binding to triple-stranded DNA is a general property of recA-like recombinases.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Alfred Nordheim
ZMBH
In Neunheimer Feld
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Federal Republic of Germany

VI. Publications:

none in the reporting period

Title of the project no.:

4. Development of general methods for the identification of proteins involved in DNA recombination and repair.

Head(s) of project:

Dr. Marco Bianchi
Dr. Giampiero Sironi

Scientific staff:

Dr. Marco Bianchi

I. Objectives of the project:

Identification of mammalian proteins involved in DNA recombination and repair.

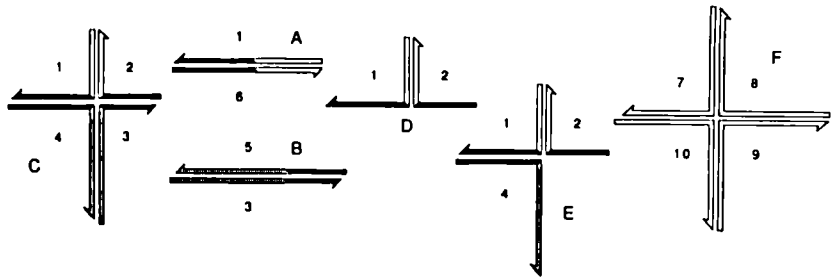
II. Objectives for the reporting period:

The purification of the rat liver protein that recognizes cruciform DNA and the production of antibodies against it.

III. Progress achieved:

METHODOLOGY

We have devoloped a powerful assay aimed at identifying a class of proteins involved in DNA recombination and repair, those recognizing Holliday junctions. We constructed artificial Holliday junctions by annealing four chemically synthesized oligonucleotides in four-way branched DNAs (molecules C and F). The artificial junction C is labeled with 32P and used as probe in gel retardation experiments. We also built control double-stranded molecules (A and B) that contain the same DNA sequences present in the cruciform molecule, but are linear rather than cruciform, and other control molecules (D and E).



We previously detected a protein from rat liver extracts that binds to molecules C and F, but not to control molecules. Binding is thus structure-specific, but sequence-independent. We conjugated molecule F to Sepharose beads, and used this resin to purify the cruciform-binding protein by affinity chromatography.

RESULTS

The cruciform binding protein was purified to physical homogeneity by conventional chromatographic techniques, affinity chromatography and electrophoresis. We obtained two polypeptides of 23 and 21 kD that showed strong cruciform-binding activity. The larger polypeptide was partially hydrolysed with trypsin, and four of the resulting oligopeptides were sequenced with a gas-phase sequenator. Each of the four sequences corresponded to stretches of the previously determined sequence of rat High Mobility Group 1 protein (HMG1). To confirm this identification, we established that antibodies raised against HMG1 protein reacted with the protein contained in the complex with cruciform DNA, resolved by electrophoresis. Finally, we transcribed and translated

in vitro the cDNA clone for rat HMG1, and verified that the protein thus synthesized did bind to cruciform DNA, but not to the linear control duplex DNAs.

DISCUSSION

We have conclusively demonstrated that HMG1 protein binds specifically to cruciform DNA. HMG1-like proteins are present in the nuclei of all eukaryotes and are apparently essential for cell viability, but their function has not been identified unequivocally. Our results imply that whenever DNA adopts a cruciform conformation, the high concentration of HMG1 in the nucleus can drive the equilibrium towards the formation of a protein-DNA complex. The physiological significance of HMG1 binding to cruciform DNA is at present unknown, but profounds effects on recombination, replication and transcription are expected.

IV. Objectives for the next reporting period:

The study of the mechanics of HMG1 binding to cruciform DNA.

The identification of proteins endowed with similar binding properties in other organisms.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Riccardo Cortese

EMBL

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D-6900 Heidelberg

Federal Republic of Germany

VI. Publications:

Bianchi M.E.
Interaction of a protein from rat liver nuclei with cruciform DNA.
EMBO J. 7, 843-849 (1988).

Bianchi M.E., Beltrame M. and Paonessa G.
Specific recognition of cruciform DNA by HMG1 protein.
Science, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-E-141-NL

Erasmus University Rotterdam
Dept. of Cell Biology and Genetics
P.O. Box 1738
NL - 3000 DR Rotterdam

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. D. Bootsma
Dept. of Cell Biology and Genetics
Erasmus University Rotterdam
P.O. Box 1738
NL - 3000 DR Rotterdam

Telephone number: 010-408 7186

Title of the research contract:

The genetic and biochemical basis of radiation sensitivity in human and other mammalian cells in culture.

List of projects:

1. Isolation and characterization of DNA repair genes in mammalian cells.

Title of the project nr.: 1

Isolation and characterization of DNA repair genes in mammalian cells.

Head(s) of project:

Dr. J.H.J. Hoeijmakers, Dr. A. Westerveld, Prof.Dr. D. Bootsma

Scientific staff:

Dr. M. van Duin, Dr. N.G.J. Jaspers, Dr. W.J. Kleijer, Drs. M. Koken, Drs. C. Troelstra, H. Odijk, J. v.d. Tol, W. Vermeulen, J. de Wit.

I. Objectives of the project:

1) The isolation, collection and genetic and biochemical characterization of radiation sensitive human and rodent cell lines.
2) Cloning of human genes involved in the genetic control of DNA repair processes by introduction of human DNA into radiation sensitive human and rodent cell lines.
3) Comparison of DNA repair genes in different species by
   a) introduction of E.coli yeast repair genes into mammalian cells and
   b) analysis of homology of DNA and aminoacid sequences of different organisms, based on conservation during evolution.

II. Objectives for the reporting period:

1. Continuation of the isolation, collection and genetic and biochemical characterization of radiation sensitive human (mainly XP and AT) and rodent cell lines (CHO and V79 mutants; in collaboration with group of Dr. Simons, (Leiden). 2. Continuation of the molecular characterization of the ERCC-1 gene with emphasis on expression of the gene: a) purification of the ERCC-1 protein (probably from a human source) and functional characterization (also involving site directed mutagenesis; b) characterization of the recently discovered antisense ERCC-1 gene; c) isolation of monoclonal antibodies directed against (parts of) the ERCC-1 protein. 3. Cloning and characterization of human genes correcting the defects in CHO UV-sensitive mutants of complementation group 3 and 6 (in collaboration with group of Van der Eb, Leiden). 4. Cloning of Drosophila genes homologous with yeast RAD repair genes.

III. Progress achieved:

1. Isolation and characterization of DNA repair mutants

A permanent cell line (XP44RO(MEL)) was established from a metastatic melanoma from an XP-C patient. As far as we know this is the first established line derived from an XP-tumor. The tumorigenic properties were related to an N-ras oncogene, activated in codon 61 by a T→A or G transversion at a potential site for dimer formation. This cell line should prove useful for studying the mechanism of excision repair, and the relationship between the XP defect and carcinogenesis.

From a number of European countries skin specimens were received taken from patients with a clinical suspicion of an inherited disorder involving mutagen hypersensitivity. Cell cultures were established from 16 patients and characteristic biochemical features were investigated in order to arrive at a final diagnosis. Out of 6 cases submitted on the suspicion of xeroderma pigmentosum or trichothiodystrophy 3 were found defective using tests of excision repair and/or daughter-strand repair. Three out of ten patients suspect for ataxia telangiectasia or the Nijmegen Breakage Syndrome were found to be affected by measuring the inhibition of DNA synthesis after X-ray exposure of the cells. In addition, prenatal diagnostic tests were performed on chorionic villi and/or amniotic fluid cells in 4 pregnancies at risk of these disease conditions.

Genetic complementation analysis of ataxia telangiectasia and the biochemically related Nijmegen Breakage Syndrome was continued and extended in collaboration with Dr. R.A. Gatti (Univ.California, Los Angeles). On the basis of these results the localization on human chromosome 11q22-23 could be established for the ataxia telangiectasia gene from complementation group AB. This is the first chromosomal assignment of a human inherited disease with hypersensitivity to radiation.

2. Isolation of repair genes by DNA transfection

a. Molecular characterization of the human DNA excision repair ERCC-1 gene and gene product.

From detailed analysis of the ERCC-1 gene region 3, interesting features emerged: 1. The presence of an antisense overlapping other gene, encoding a 2.6 kb mRNA with a 3′ terminus complementary to that of ERCC-1 (see report 1987). We have found this exceptional type of gene configuration also in the mouse ERCC-1 locus. Independently, Dr. Prakash and coworkers (Rochester) discovered that the yeast RAD10 gene harbours a 3′ overlapping antisense gene as well. These findings indicate that antisense transcription in the ERCC-1/RAD10 gene regions represents an evolutionary conserved feature with an important function and extends the homology between the two genes to their gene organization.

2. In collaboration with Dr. McGinnis (Los Alamos) a CpG island was discovered in the ERCC-1 promoter region.

3. The ERCC-1 gene region appeared to reside in a very interesting area of human chromosome 19. It is located on the same 250 kb Not-1 restriction fragment as ERCC-2 and the gene for creatine kinase (muscle type). Furthermore, based on genetic evidence it is very close to the locus causing the hereditary disorder dystrophia myotonica, for which

no probe is available (in collaboration with Dr. B. Wieringa, Nijmegen).

Site directed mutagenesis of ERCC-1 has shown that the C-terminal 12 aminoacids (in the region with homology to the C-terminus of uvrC) are still essential for its function. Similar experiments focussed on the postulated DNA binding domain are in progress.

Since our efforts to overproduce ERCC-1 as a free protein in E.coli have failed (possibly because of rapid proteolytic degradation) 2 alternative sources for overproduction and purification of the ERCC-1 protein have been explored:

1. Amplification of the transfected ERCC-1 gene in CHO cells using a cotransfected DHFR gene and selection by increasing methotrexate concentration. Although a >100 x amplification of ERCC-1 was achieved at the DNA and RNA level, only a relatively small increase at the protein level was found, as shown by Western blotting using polyclonal antibodies against the ERCC-1 protein. We have started pilot experiments to purify the ERCC-1 protein from these cells or HeLa.
2. Dr. S. Prakash (Rochester) has constructed yeast strains which harbour the ERCC-1 gene in a high expression vector. After verification that the ERCC-1 expression levels are sufficiently high we will use those strains as an additional source for the ERCC-1 protein.

b. Isolation and characterization of the ERCC-3 gene (in collaboration with G. Weeda and A.J. van der Eb, Leiden University).

Using excision repair deficient mutant 27-1 of CHO complementation group 3 (kindly provided by Dr. R. Wood, London) the human ERCC-3 gene was cloned. With the aid of unique probes, containing conserved sequences, cDNA clones were isolated, which corrected the repair defect (UV-sensitivity) of 27-1 with a very high efficiency. Characterization and functional analysis of the gene and cDNA revealed a.o. the following features:

The gene is approximately 35 kb and assigned to chromosome 2q21. It encodes a mRNA of 3 kb. ERCC-3 is probably not involved in XP-A, -C, -D, -E, -G, -H and -I (XP-B and -F not tested).

The cDNA encodes a predicted protein of 782 amino acids, with putative nucleotide binding, helix turn-helix DNA binding, nuclear location signal and histon binding domains. The ERCC-3 amino acid sequence bears no significant homology to known repair genes of yeast and E.coli.

c. Isolation and characterization of the ERCC-6 gene

The moderately UV-sensitive mutant UV61 (complementation group 6, generously provided by Dr. Thompson, Livermore) was utilized to generate UV-resistant transformants, after introduction of human genomic DNA. From a lambda library, constructed from the DNA of a secondary transformant, the entire human insert (approximately 125 kb, notably poor in repeat sequences) was cloned and mapped. By 'coinheritance' analysis of the DNA of independent other transformants we deduce that the cloned ERCC-6 gene probably has a size of $\sim$100 kb. Thusfar, a partial cDNA (3.1 kb) has been isolated.

3. Isolation of repair genes by sequence homology

The high sequence conservation between the cloned human and yeast excision repair genes, analyzed thusfar (ERCC-1, -2 and -3) prompted us to attempt isolation of human genes by virtue of cross-hybridization with yeast repair genes. Using as a probe the Saccharomyces cerevisiae RAD6 gene (encoding a ubiquitin conjugating enzyme involved in DNA damage induced mutagenesis) we have isolated and sequenced the RAD6 genes of Schizosaccharomyces pombe (a very distantly related yeast species) and of Drosophila melanogaster. The S.pombe gene, which shared 76% identity at the amino acid level with S.cerevisiae RAD6, appeared to lack the sequence specifying the very acidic tail of the S.cerevisiae protein. The Drosophila gene shares 69% identity and probably also does not encode a acidic C-terminus. Using the Drosophila and yeast RAD6 probes we will try to isolate the homologous human gene and study its function, expression and possible involvement in human repair disorders. Similar experiments for other RAD gene (e.g. RAD1) are ongoing.

IV. Objectives for the next reporting period:

1. Continuation of the isolation, collection and genetic and biochemical characterization of radiation sensitive human (mainly XP and AT) and rodent cell lines (CHO and V79 mutants; in collaboration with group of Dr. Simons, (Leiden). 2. Continuation of molecular and functional characterization of the ERCC-1 gene and gene product in in vivo assay systems. 3 Molecular characterization of the ERCC-3 gene and isolation of the ERCC-3 protein. Cloning of the ERCC-6 cDNA and molecular characterization of this gene. 4. Cloning of the human RAD 6 and RAD 1 homologous genes and of the Drosophila and yeast ERCC-3 homologous genes.

V. Other research group(s) collaborating on this project ((names and address(es)):

- the Department of Molecular Carcinogenesis, University of Leiden (Prof.Dr. A.J. van der Eb)
- the Department of Molecular Genetics, University of Leiden (Prof.Dr. P. v.d. Putte).
- the Department of Radiation Genetics and Environmental Mutagenesis, University of Leiden (Prof.Dr. P.H.M. Lohman, Dr. A.A. van Zeeland and Dr. J.W.I.M. Simons).
- the Medical Biological Laboratory TNO, Rijswijk (Dr. G.P. v.d. Schans).
- the MRC Cell Mutation Unit, University of Sussex, England. (Prof.Dr. B.A. Bridges).

VI. Publications

van Duin M, Janssen JH, de Wit J, Hoeijmakers JHJ, Thompson LH, Bootsma D, Westerveld A. Transfection of the cloned human excision repair gene ERCC-1 to UV-sensitive CHO mutants only corrects the repair defect in complementation group 2 mutants. Mut Res 1988;193:123-30.

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Zdzienicka M, Jaspers NGJ, van der Schans GP, Natarajan AT, Simons JWIM. Ataxia telangiectasia-like Chinese hamster V79 cell mutants with radioresistant DNA synthesis, chromosomal instability and normal DNA strand break repair. Cancer Res 1989; (in the press).

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Duin van M, Vredeveldt G, Mayne LV, Odijk H, Vermeulen W, Klein B, Weeda G, Hoeijmakers JHJ, Bootsma D, Westerveld A. The cloned human DNA excision repair gene ERCC-1 fails to correct xeroderma pigmentosum complementation groups A through I. Mutat Res 1989; (in the press).

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2. Thesis

Van Duin M. Cloning and characterization of the human DNA excision repair gene ERCC-1. Thesis Erasmus University Rotterdam 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-E-142-UK

Head(s) of research team(s) [name(s) and address(es)]:

Prof. B.A. Bridges
MRC Cell Mutation Unit
University of Sussex
Falmer
GB - Brighton BN1 9RR

Telephone number: 0273-678123

Title of the research contract:

The genetic and biochemical basis of radiation sensitivity in cultured human and other mammalian cells.

List of projects:

1. Isolation and characterization of DNA repair genes.
2. DNA repair and mutagenesis.

Title of the project no.:

1. Isolation and characterisation of DNA repair genes

Head(s) of project:

Dr M. H. L. Green

Scientific staff:

Dr C. F. Arlett
Professor B. A. Bridges
Dr J. Cole
Dr S. W. Dean
Dr A. R. Lehmann

I. Objectives of the project:

To provide an understanding of the modes of action of DNA repair genes in order to assess their contribution to the response of human cells to DNA damage and to ionising radiation in particular

II. Objectives for the reporting period:

(a) To maintain expansion of the culture collection

(b) To continue the cellular immortalisation programme

(c) To study loss of O^6-alkyltransferase during the process of immortalisation

(d) To maintain progress towards the molecular cloning of the Cockayne's syndrome and 46BR genes

(e) To develop alternative strategies for transfer of resistance genes to human cells

(f) To compare the radiation response of T-lymphocytes and fibroblasts and to analyse their reproducibility

III. Progress achieved:

(a) *Expansion of the cell culture collection*

Additional cultures have been accumulated into the collection from normal individuals, patients suffering or suspected as suffering from DNA repair defective diseases and from their parents. 33 skin biopsies have been converted into primary fibroblast cultures. 28 fibroblast cultures have been received from other laboratories. 80 blood samples have been collected, mononuclear cells separated and stored in liquid nitrogen. T-cell lines have been generated from 11 of these bloods.

Our collection is recognised internationally as a significant resource. During the year under review a total of 98 fibroblast cultures were dispatched in 36 different shipments: 17 within the U.K., 10 to Europe, 8 to the USA and 1 to Japan.

(b) *Cellular Immortalization Programme*

We have initiated our standard immortalization procedure which takes approximately one year for the establishment of a proven immortal culture on cells from 7 normal donors, 1 trichothiodystrophy and one Fanconi's anaemia. From these, we have already 1 completely immortalised line, as well as 12 independent sets of frozen pre-crisis material available for experimentation. In addition, 2 lines, initiated before this reporting period have now come through crisis.

(c) *Loss of O^6-alkyltransferase during the process of immortalisation*

Our studies on the loss of methyltransferase suggest that it occurs, in conjunction with neither initial transformation nor escape from crisis, but at some random time between these two events. The Mex^- phenotype appears able to spread through a growing pre-crisis population with remarkable rapidity. We are currently investigating whether the conversion of a pre-crisis population to Mex^- is explicable in terms of a selective advantage of Mex^-, or whether another explanation must be sought. We have also confirmed that resistance to N-methyl-N-nitrosourea can occur without recovery of methyltransferase and that this mechanism confers cross-resistance to 6-thioguanine and hypersensitivity to the chloroethyl-nitrosourea mitozolomide.

(d) *Progress towards the molecular cloning of Cockayne's syndrome and 46BR genes*

Secondary transfectants have remained recalcitrant in response to our attempts to maintain them in culture and this project has been suspended to await the outcome of alternative strategies for cloning (see (e) below).

(e) *Alternative strategies for cloning of human DNA repair genes.*

We have commenced a project using the fission yeast *Schizosaccharomyces pombe* as a tool for cloning human DNA repair genes. Two approaches are being adopted:-

(i) Many radiation-sensitive mutants of *S. pombe* were isolated in the 1970's These mutants fall into 22 different complementation groups. Cloning of the genes which are deficient in these mutants has commenced, and we have already succeeded in cloning the *S. pombe rad4* gene. When a family of *S.*

pombe DNA repair genes has been cloned, their sequences will be compared with those of genes which have been cloned in other laboratories from *S. cerevisiae*. Such a comparison will enable us to identify important conserved sequences, from which we will design oligonucleotides, with which we will probe human gene libraries. This strategy should enable us to identify, clone and characterise some of the homologous human genes.

(ii) Human gene expression libraries under the control of the SV40 promoter are expressed in *S. pombe*. By transfection of a cell-cycle mutant of *S. pombe* with such a human library Lee and Nurse have been able to clone a human cell cycle-controlling gene. We propose to use an analogous approach, using the *S. pombe* radiation-sensitive mutants as recipients.

(f) *The radiation responses of T-lymphocytes and fibroblasts and analysis of their reproducibility*

We have now accumulated a substantial data base for cell survival parameters using fibroblasts and T-lymphocytes, in some instances from the same individual (Arlett *et al.*, 1988; Cole *et al.*, 1988).

Studies with 22 primary fibroblast cultures show that: (i) for six normal strains values of Do vary from 1.30-1.51 Gy, (ii) the radiosensitivity of ataxia-telangiectasia (A-T) cell strains is confirmed. 8 cultures gave Do values of 0.39-0.71 Gy and (iii) the A-T heterozygotes fell close to the normal range, 6 cultures gave Do values of 1.21-1.52 Gy.

Cells which had been transformed (16 cultures) with either whole SV40 virus or with the plasmid pSV3 using the selectable markers *gpt* or *neo* all showed enhanced radioresistance. Transformation enhanced radiation resistance whether or not the immortalisation step had taken place (see b).

T-lymphocytes have also been examined for radiosensitivity. *The rapidity with which estimates of cellular radiosensitivity may be made with T-lymphocytes (9-14 days) may not only have important implications for radiation protection but may also allow an assessment of an individual's radiation response prior to a course of radiotherapy.* A substantial validation study is in progress to establish the reproducibility of the assay and any correlation with the radiation sensitivity of other cell types. The T-lymphocytes have been studied as a population of non-dividing cells separated from whole blood. Lymphocytes from 9 normal individuals gave a mean Do value of 1.55 Gy but unlike fibroblasts they appear to have a genuine but small shoulder component in the survival curve. 7 A-T patients gave a mean Do value of 0.73 Gy and the heterozygotes 1.32 Gy. Cells derived from cord blood (8 bloods mean Do 1.24 Gy) appear to be more radiosensitive than from normal adult donors, an observation which requires urgent confirmation. In addition T-lymphocyte lines have been established from 9 normal individuals and 2 A-T patients. These cells are more radiosensitive than the Go T-lymphocytes and show a reduction in the extent of shoulder. The distinction between A-T and normals is maintained.

IV. Objectives for the next reporting period:

(a) To maintain expansion of the culture collection
(b) To continue the cellular immortalisation programme
(c) To continue to analyse the radiation responses of T-lymphocytes and fibroblasts
(d) To use 6-thioxanthine to select against *gpt* in lines immortalised with pSV3 *gpt*
(e) To continue to study loss of O^6-methyltransferase and other events associated with immortalisation
(f) To clone and characterise DNA repair genes from *Schizosaccharomyces pombe*
(g) To examine the feasibility of correcting the *rad4* defect in *S. pombe* with human DNA

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Department of Genetics, Erasmus University, Rotterdam

Department of Biochemistry, University of Leiden

Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden

Medical Biological Laboratories, TNO, Rijswijk

VI. Publications:

Arlett, C. F. (1987) The radiosensitivity of cultured human cells. *Radiation Research, Volume 2, Proceedings of the 8th International Congress of Radiation Research, Edinburgh, July 1987, Fielden, E. M., Fowler, J. F., Hendry, J. H. and Scott, D., Eds., Taylor and Francis, London* pp424-430.

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Cole, J., Arlett, C. F., Green, M. H. L., Harcourt, S. A., Priestley, A., Henderson, L., Cole, H., James, S. E., and Richmond, F. (1988) Comparative human cellular radiosensitivity: II. The survival following gamma-irradiation of T-lymphocytes, T-lymphocyte lines, lymphoblastoid cell lines and fibroblasts from normal, ataxia-telangiectasia patients and heterozygotes. *International Journal of Radiation Biology* **54**, 929-943.

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Green, M. H. L., and Lowe, J. E. (1987) Failure to detect a DNA repair-related defect in the transfection of ataxia-telangiectasia cells by enzymatically restricted plasmid. *International Journal of Radiation Biology* **52,** 437-446.

Hilgers, G., Abrahams, P. J., Schouten, R., Cornelis, J. J., Lehmann, A. R., Van Der Eb, A. J., and Rommelaere, J. (1987) Les cellules de patients atteints d'ataxia telangiectasia manifestent une capacite normale de reactivation radioinduite du virus HSV-I endommage. *C. R. Soc. Biol.* **181,** 432-438.

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Lehmann, A. R., Arlett, C. F., Broughton, B. C., Harcourt, S. A., Steingrimsdottir, H., Stefanini, M., Taylor, A. M. R., Natarajan, A. T., Green, S., King, M. D., Mackie, R. M., Stephenson, J. B. P., and Tolmie, J. L. (1988) Trichothiodystrophy, a Human DNA Repair Disorder with Heterogeneity in the Cellular Response to Ultraviolet Light. *Cancer Res.* **48,** 6090-6096.

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Pippard, E. C., Hall, A. J., Barker, D. J. P., and Bridges, B. A. (1988) Cancer in homozygotes and heterozygotes of ataxia-telangiectasia and xeroderma pigmentosum in Britain. *Cancer Res.* **48,** 2929-2933.

Title of the project no.:

2. DNA Repair and Mutagenesis

Head(s) of project:

Dr. C. F. Arlett

Scientific staff:

Professor B. A. Bridges
Dr. J. Cole
Dr. A. R. Lehmann

I. Objectives of the project:

To obtain a detailed understanding of the mechanisms of mutagenesis in mammalian (especially human) cells in order (a) to relate DNA repair to mutagenesis and to different aspects of human health and (2) to develop new mutagenesis systems.

II. Objectives for the reporting period:

(a) Production and analysis of radiation induced mutants in (i) the *lacZ* gene of EBV shuttle vectors maintained in normal and repair-deficient lines and (ii) the *supF* gene of the shuttle vector pZ189 passaged through normal and repair deficient lines.

(b) Molecular analysis of hprt mutants by measurements of HPRT and hprt mRNA activity, Southern analysis of the hprt gene and analysis of point mutations using the polymerase chain reaction.

(c) Molecular analysis of T-cell receptor (Ti) alpha and beta chain genes in mutant lymphocytes.

III. Progress achieved:

(a) *Mutations in shuttle vectors*

(i) The EBV-based shuttle vector described previously has now been introduced into several normal, XP, A-T and CS cell lines and in almost all cases the plasmid is maintained extrachromosomally. The spontaneous mutation frequency in cell lines studied is less than 10^{-5}, and three UV induced mutants in normal cells have been analysed by sequencing. Surprisingly all three contained deletions.

(ii) Because of the difficulty involved in generating sufficient numbers of mutants with the EBV-shuttle vectors, we have begun a study using the SV40-based vector pZ189. Although this is a transient replication system, the plasmid replicates to a high copy number in SV40-transformed human cells and large numbers of mutants in the *supF* gene on the plasmid can be easily generated. The spontaneous mutation frequency is about 10^{-4} and many mutant plasmids have been induced by UV-irradiation of pZ189, followed by passage through normal, A-T or CS cells. About 200 mutant plasmids have been isolated and twenty-five have been sequenced so far, in order to build mutational spectra for the different cell lines.

(b) *Molecular analysis of hprt mutants in human cells*

(i) T-lymphocytes

We have carried out Southern analysis of 35 6-thioguanine-resistant mutant lymphocytes from normal and A-T donors. In no case is the *hprt* mutation attributable to a large deletion or rearrangement. PstI digestion of DNA from all mutants gave similar hprt-hybridising bands to those in non-mutants. We are now developing the polymerase chain reaction (PCR) and direct sequencing techniques for the molecular analysis of point mutations.

(ii) Cultured Fibroblasts

We are carrying out similar analyses on spontaneous and UV-induced mutations at the *hprt* locus in SV40-transformed normal and XP fibroblasts. Many mutants have been generated and expanded. Southern analysis of spontaneous mutants from the normal cell line has revealed deletions in a substantial proportion of the mutant cells. Deletions covering both part and the whole of the gene have been detected.

(c) *Molecular analysis of T-cell receptor beta and gamma chain genes in mutant T-lymphocytes*

In order to assess the origin of the hprt$^-$ T-lymphocytes, Southern blots of their DNA have been hybridised with T-cell receptor beta and gamma chain probes. If the mutant T-cells from the same patient are of independent post-thymic origin, they should contain different rearrangements of the T-cell receptor genes. Different rearrangements have been found for most of the T-cell clones analysed so far. This indicates that the mutant clones from any one individual have in general arisen from independent mutations rather than from clonal expansion of a single mutant cell.

(d) *Trichothiodystrophy (TTD) - a new DNA repair defective syndrome*

Studies of the cellular survival, mutation and DNA repair characteristics of this disease are revealing considerable heterogeneity. Some individuals with TTD appear to have the same radiosensitivity and the same genetic defect as in xeroderma pigmentosum complementation group D. The fact that despite this they do not develop skin cancer, may have profound implications fopr our understanding of carcinogenesis in general and for sunlight-induced cancer in particular.

IV. Objectives for the next reporting period:

(a) To obtain comparative UV-induced mutation spectra in the supF gene of pZ189 for normal, A-T and CS cells in order to determine if DNA repair deficiencies affect the quality and/or the quantity of the mutations produced.

(b) To continue the molecular analysis of hprt mutations induced by UV in normal and repair-deficient human fibroblasts using both Southern analysis and the PCR technique.

(c) To undertake analysis of T-cell receptor beta and gamma chain genes in mutant T lymphocytes.

(d) To investigate the molecular defects in DNA repair in trichothiodystrophy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Department of Genetics, Erasmus University, Rotterdam.

Department of Biochemistry, University of Leiden.

Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden.

Medical Biological Laboratory, TNO, Rijswijk.

VI. Publications:

Henderson, L., Cole, H., Cole, J., James, S. E., and Green, M. H. L. (1986) Detection of somatic mutations in man: evaluation of the microtiter cloning assay for T-lymphocytes. *Mutagenesis* **1,** 195-200.

Lehmann, A. R. (1987) Cockayne's syndrome and trichothiodystrophy: defective repair without cancer. *Cancer reviews* **7,** 82-103.

MacGregor, G. R., and Burke, J. F. (1987) Stability of a bacterial gene in a bovine papillomavirus-based shuttle vector maintained extrachromosomally in mammalian cells. *J. gen. Virol.* **68,** 247-252.

MacGregor, G. R., James, M. R., Arlett, C. F., and Burke, J. F. (1987) Analysis of mutations occurring during replication of a SV40 shuttle vector in mammalian cells. *Mutation Res.* **183,** 273-278.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-294-UK

University of St. Andrews
Dept. of Biology & Preclinical Med.
College Gate
GB-KY16 9AJ - Fife, SCOTLAND

Head(s) of research team(s) [name(s) and address(es)]:

Dr. P.E. Bryant
Dept.of Biology & Preclinical Med.
University of St. Andrews
College Road
GB-KY16 9AJ - Fife, SCOTLAND

Telephone number: 334-76161 Ext.7104

Title of the research contract:

Molecular mechanisms of radiation damage to chromosomes of human and rodent cells.

List of projects:

Molecular mechanisms of radiation damage to chromosomes of human and rodent cells.

Title of the project no.:

Molecular mechanisms of radiation damage to chromosomes of human and rodent cells.

Contract: B16.0294.UK(H).

Head(s) of project:

Dr. P.E. Bryant

Scientific staff:

I. Objectives of the project:

To gain an understanding of the mechanisms involved in the induction of chromosomal aberrations in cells exposed to ionizing radiation. The work will focus particularly on the biochemical mechanisms of formation of exchange-type chromosomal aberrations in G_o and G_1 rodent and human cells.

II. Objectives for the reporting period:

To establish conditions for the optimum maintenance of rodent and human fibroblastic lines in the G_o and G_2 phases. Measurement of the length of G_2 phase using ^{3}H-thymidine labelling. Measurement of kinetics of break rejoining in G_2 cells. Measurement of repair of DNA double-strand break repair using neutral filter elution and DNA-unwinding methods.

III. Progress achieved:

1. Methodology

1.1 Measurements of kinetics of chromatid aberrations in human lymphocytes and in normal and AT fibroblasts.

Peripheral blood was taken from healthy normal donors and cultured in RPMI (1640) medium supplemented with 15% FCS and buffered with HEPES (20 mmol/l). Lymphocytes were stimulated with PHA and used after 72h in culture. G_2 cells were scored by harvesting at between 1.5 and 4.5 hours following X-irradiation. Cultures were irradiated in medium with X-rays (250 Kv, 14 mA, 0.5 mm Cu) at a dose-rate of 0.8 Gy/min. The X-ray dose in all experiments was 0.75 Gy. Parallel cultures were treated with ara A and ara C (Sigma) added to various final concentrations. Cells were arrested with colcemid and metaphase spreads made by standard cytogenetic procedures. A series of seven experiments have so far been completed. Fibroblasts were cultured as previously described (Mozdarani and Bryant, 1989, Int. J. Radiat. Biol., 55, 71) and the kinetics of aberrations examined in G_2 cells in the presence or absence of ara C at between 1.5 and 3.5h before fixation. Irradiation was as above with a dose of 1 Gy.

1.2 Neutral filter elution.

Neutral filter elution has been used to measure repair of DNA dsb in 3H-TdR labelled CHO cells. Elution was performed as per Bradley and Kohn except that the pH value of the elution solution has been varied between 7.2 and 9.6. Also, before elution was begun, cell lysates were treated with proteinase k or pronase at 60°C for 1h. The dose used was 30 Gy.

1.3 Labelled mitoses using autoradiography.

The length of G_2 phase was measured in CHO cells using ^{3}H-TdR labelling. Samples were grown overnight at 37°C from 2.10^5 cells per 25 cm^2 flasks and then duplicate samples shifted to either 33°C or 29°C for 3h before addition of ^{3}H-TdR (5 µCi/ml). Cells were harvested at intervals of up to 7h, washed

free of label and fixed by standard procedures. Slides were prepared and dipped in autoradiographic emulsion (Ilford K2), dried and held in darkness for 4 days. Slides were then developed, stained in Giemsa and the frequencies of labelled mitoses scored. Mitotic figures were scored as labelled if they contained 20 or more grains.

2 Results

Figs 1a and 1b show kinetics of rejoining of chromatid breaks in X-irradiated human lymphocytes treated with ara A or ara C. Kinetics of rejoining in untreated (X-irradiated) cells was found to be first-order with a t 1/2 of 54 minutes. Ara A at 100 or 200 µmol/l had no effect on these kinetics; however, at 800 µmol/l a strong inhibition was observed. In contrast, ara C even at very low concentrations (e.g. at 1.5 µmol/l) was found to increase the number of deletions and to inhibit repair. By 40 µmol/l a flat response was obtained, indicating complete inhibition of rejoining. No exchange aberrations were observed under any of these conditions. The length of G_2 in stimulated lymphocytes was determined by PCC kinetics to be approximately 4.5 h. Experiments with normal and AT fibroblasts in the absence of ara C yielded similar results to those previously obtained in these lines with the rejoining of breaks a first-order function of time and a half-time of approximately 2.4 h (data not shown). In both lines the effect of ara C (100 µmol/l) was to increase the number of deletions with time by factors of approximately 2 (normal) and 1.5 (AT) over a two hour period between irradiation and fixation. Exchanges increased with time in both lines but ara C led to a relatively small increase (1.6) over control values in contrast to the three fold increase produced by ara A (Mozdarani and Bryant, 1987, Mutagenesis, 2, 371). Labelled mitosis curves for CHO K1 cells held at 37°C, 33°C and 29° showed that the G_2 phase was lengthened as temperature decreased. The length of G_2 was estimated to be 3h at 37°C, 5h at 33°C and 7h at 29°C. Neutral filter elution performed on CHO cells showed linear induction kinetics at pH 7 and an S-shaped induction curve at pH 9.6.

Repair of dsb as measured by the change in relative elution was rapid (t 1/2 of approximately 15 mins) and kinetics were similar at both pH 9.6 and 7.4.

3 Discussion

Previous work with G_2 normal human and ataxia telangiectasia (AT) fibroblasts (Mozdarani and Bryant 1987; 1989. Int. J. Radiat. Biol., 55, 71) has shown that rejoining of chromatid deletions is first order in both lines, with a t 1/2 of approximately 2.5 h, a value which was confirmed in the present experiments. The frequencies of exchanges in both normal and AT fibroblasts were shown to increase with time, and in the presence of ara C the frequency increased by approximately 1.6 in both lines. It has been suggested that this indicates the presence of two separate mechanisms, one for rejoining breaks (presumably reflecting underlying dsb repair) and another for misjoining breaks to form exchanges.

We have now also investigated the kinetics of chromatid aberrations in G_2 normal human lymphocytes. Preston (1980, Mut. Res., 69, 71) had suggested that in lymphocytes ara C inhibition of dsb repair allowed base damage incision to occur leading to additional chromatid breaks and that exchanges would be inhibited from forming while dsb rejoining was inhibited. Our data (figs 1a and 1b) show that the rejoining of breaks approximated to first-order kinetics (as in fibroblasts), but with a t 1/2 of about 1h. This was 2-3 times the rate than found in fibroblasts under similar conditions, although the initial numbers of breaks per chromosome were almost the same in the two different cell types. In the presence of ara A and ara C (figs 1a and b) inhibition of break rejoining occurred, but in the case of ara A only at higher concentrations than used to inhibit rejoining in fibroblasts. No exchanges were observed, a result which agrees with those of Preston (1980). It might be argued that the lack of exchanges found could be due to the more rapid disappearance of breaks, however, even when break rejoining was inhibited (e.g. 800 µmol/l ara A) no exchanges were observed. These observations confirm our view

that the mechanism for rejoining breaks (reflecting dsb) is quite separate from that for misjoining. In experiments with rodent (CHO) cells we have investigated the possibility of altering the cell cycle kinetics so as to lengthen G_2 phase. Using the ^{3}H-thymidine labelled mitoses method we have established that the G_2 phase lengthens from 3h to 5h when the temperature is shifted from 37°C to 33°C and to 7h at 29°C with a relatively small change in mitotic index. Experiments are in progress to study the rejoining of breaks and the formation of exchanges in CHO cells at these lowered temperatures. By lengthening G_2 phase it should be possible to study the kinetics of rejoining and exchange formation in greater detail than is possible at 37°C where the total time available to "view" kinetics is 3h or less. This is in contrast to the G_2 phase in human cells which is 4-5h in length. In parallel we will study the effect of temperature and the inhibitors ara A and C on repair of dsb using neutral elution.

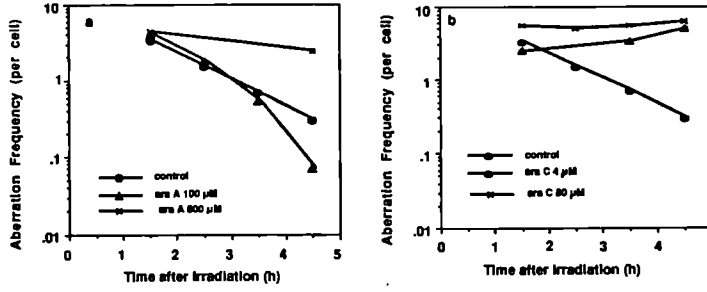

Figure 1. Frequencies of chromatid aberrations (deletions and gaps) per cell as a function of time between X-irradiation (0.75 Gy) and fixation in normal human blood lymphocytes stimulated with PHA. a) Cells held in the presence or absence of a) ara A and b) ara C between irradiation and fixation.

IV. Objectives for the next reporting period:

(a) Measurements will be made of the kinetics of rejoining of chromatid breaks and formation of exchanges in G_2 CHO cells at 37°C, 33°C and 29°C.
(b) Using PCC, kinetics of rejoining of breaks and formation of exchanges in G_0 CHO cells will also be measured.
(c) The feasibility of making similar measurements in Chinese hamster lymphocytes will also be investigated. This will provide valuable data to compare with our human lymphocyte data.
(d) Measurements of DNA dsb rejoining will be made in CHO and human cell lines at various temperatures and in presence of inhibitors.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-E-143-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. B.M. Cattanach
Radiobiology Unit, Genetics Div.
Medical Research Council
Harwell, Didcot
GB - Oxon OX11 ORD

Telephone number: 0235-834393

Title of the research contract:

Mutation studies upon spermatogonial stem cells of mammals and genetic tests for non-disjunction in the mouse.

List of projects:

1. Experimental studies of non-disjunction in the mouse.
2. Factors affecting the yield of mutations from spermatogonial stem cells of mammals.

Title of the project no.: 1. Experimental studies on non-disjunction in the mouse

Head(s) of project: Dr. B.M. Cattanach F.R.S.

Scientific staff: Dr. B.M. Cattanach F.R.S.

I. Objectives of the project:

To develop genetic methods for detecting non-disjunction and chromosome loss in mice.

II. Objectives for the reporting period:

1. To finalise experiments to assess the effectiveness of the Robertsonian translocation, Rb(1.3)1Bnr, with both arms marked for detecting chromosome 1 and 3 non-disjunction loss using the Rb tester method.

2. To investigate using equivalent methodology the effectiveness of Rb(11.13)4Bnr with both arms marked for detecting chromosome 11 and 13 non-disjunction.

3. To estabish Rb(11.13)4Bnr and Rb(10.11)8Bnr stocks with a chromosome 11 marker in order to set up a test for non-disjunction/loss involving this chromosome using the Monobrachial Homology (MBH) test.

III. Progress achieved:

1. Methodology

1. Rb1Bnr heterozygotes with both chromosome arms genetically marked had earlier been used to screen for chromosome 11 and 13 loss following female X-irradiation at a dose of 4 Gy. The ln gene was used to mark chromosome 11 and ma to mark chromosome 3. 6 out of 2915 young exhibited the ln phenotype and from this the frequency of chromosome 1 loss was estimated to be 3.02%. No evidence of chromosome 3 loss was detected, which was surprising because chromosome 3 non-disjunction in the tester parent was known to be comparable to that for chromosome 1. Rb1Bnr has now been used to screen for the loss events following male irradiation using the same test procedures and X-ray dose.

2. The effectiveness of doubly-marked Rb(11.13)4Bnr heterozygotes for detecting chromosome 11 and 13 non-disjunction and loss events in chromosomally normal mice is being ascertained by screening for loss events, initially in males. The vt gene has been used as the chromosome 11 marker and bg as the marker for chromosome 13. Unmarked chromosomally normal males were placed with tester vt Rb4Bnr bg/vt + bg females for one week following irradiation. All progeny therefore derived from irradiated spermatozoa and to facilitate comparison of the results with those obtained with other chromosomes the 4 Gy X-ray dose was used to induce chromosome loss.

2. Results

1. 594 females were employed to screen for chromosome 11 and 13 losses in the spermatozoa of the irradiated males. Most became pregnant and produced young for phenotypic classification. The mean litter size was lower (3.90) than had been found in the previous study (5.63) using the same test system and X-ray dose in females, but was still large enough to make the test effective.

1888 young were reared to weaning age when ln and ma are normally classified. 4 ln young (0.21%) were detected which accords well with the results of the previous female study (0.22%) and all survived to adulthood when their genotypes could be verified. The frequency of chromosome 11 loss from the gametes of the irradiated males was calculated to be 2.94% (95% confidence limits, 0.88 and 6.26). Only 1 ma animal (0.05%) was detected (gametic loss 0.81%, 95% confidence limits, 0.02-3.90) which again is in accord with the female data (0%). The

difference between the ln and ma results in these experiments, as before, is statistically significant (P = 1.00).

2. The test for chromosome 11 and 13 loss events in doubly-marked Rb(11.13)4Bnr mice has not yet provided sufficient data to be informative.

3. Rb(11.13)4Bnr mice with their chromosome 11 marked with vt were already available but a stock of Rb(10.11)8Bnr animals equivalently marked with vt has now been made. Intercrosses to produce R4Bnrvt/R8Bnrvt compounds are breeding and tests for chromosome 11 loss using the MBH tester method are being initiated.

3. Discussion

Diverse radiation experiments have indicated that dominant lethality levels (attributable to chromosome breakage events) are approximately similar following the irradiation of mature oocytes and spermatozoa at doses approximating that used here (4 Gy). The lower litter size in the current experiment with irradiated males (3.90) relative that of the previous study with females (5.63) at the same dose therefore suggests that non-disjunction is higher in the heterozygous R4Bnr/+ tester females than in the equivalent tester males. This would be consistent with the results of other studies with mouse Robertsonian translocations. When allowance is made for this, the estimated frequency of chromosome 11 loss from males falls from 2.94% to 1.94% (95% confidence limits 0.56-4.40) but clearly the difference from the female value (3.02%) is not large enough to establish that X-ray induced chromosome loss from females is higher than that from males.

The near-absence of ma young in the present experiment is consistent with the results of the test on females, but the poor response remains difficult to interpret. The non-disjunction frequency for chromosome 3 in Rb1Bnr heterozygotes is known to be very similar to that for chromosome 1 and therefore the difference between the frequencies of ln and ma young in the current experiment cannot be explained on this basis. Although ma is less easily classified than ln, classification difficulties have not been experienced in any previous studies and are therefore unlikely to have been a factor here. The further possibility that chromosome 3 is less liable to damage events that would lead to its loss than chromosome 1 also seems untenable. Further investigation with another chromosome 3 gene marker may be necessary to resolve this problem.

IV. Objectives for the next reporting period:

1. To complete the studies with Rb(11.13)4Bnr using the Rb tester method.

2. To undertake the studies with Rb(11.13)4Bnr and Rb(10.11)8Bnr using the MBH tester method.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

CATTANACH, B.M. Chromosomes 1 and 3 non-disjunction in Rb(1.3)1Bnr heterozygotes. Mouse News Letter, 81: 64 (1988).

Title of the project no.: 2. Factors affecting the yield of mutations from spermatogonial stem cells of mice

Head(s) of project: Dr. B.M. Cattanach F.R.S.

Scientific staff: Dr. B.M. Cattanach F.R.S.

I. Objectives of the project:

To determine how the biology of spermatogenesis and other factors influence the mutational response to X-rays, so that mouse data can be more validly extrapolated to man.

II. Objectives for the reporting period:

1. To analyse by genetical and other means mutants of interest induced in the recently completed combined chemical-X-ray mutation experiments.

2. To investigate the sensitivity of spermatogonial stem cells of 101/H mice to killing, translocation and mutation induction by X-rays.

3. To undertake screening studies for differences between other mouse strains in their sensitivities to spermatogonial killing.

4. To investigate further the cell stage sensitivity differences of spermatogonial stem cells to specific locus and translocation induction by X-rays.

III. Progress achieved:

Results and Discussion

1. Analysis of mutations:

22 dominant mutations have been investigated. Most represented new mutations at known loci. Thus, 4 steel, 4 brachyury, 4 mottled, and 1 tabby mutation have been found. Most of the remainder were skeletal mutations at loci whose position in the linkage map is currently being sought. Of significance, is the discovery that several of the dominant mutations were deletions, some of which were large enough to be detected cytologically. This has also proven true for 2 out of 3 albino mutations detected in the same study. Each of the deletions is now being further investigated using molecular techniques.

2. 101/H sensitivity studies:

a) Comparison of the capacities of the 101/H mouse and its C3H/HeH x 101/H F_1 hybrid to repair the germinal epithelium following depletion by 4 Gy X-rays has been investigated using testis weight and sperm count as indicators of recovery. From 4-8 weeks post-irradiation both indicators showed the inbred strain to be more severely affected by the radiation but evidence of repopulation was noted to occur at the same time post-treatment in the 2 groups. There is therefore no reason to suspect that the spermatogonial stem cell kinetics of the 101/H mouse differs significantly from that of the hybrid and is responsible for the different stem cell killing and translocation responses to X-rays.

b) The sensitivity to translocation induction by X-rays of the 'triggered' spermatogonial stem cell population that survives 24 h after a priming dose of X-rays is being reinvestigated in 101/H mice, with the C3H/HeH x 101/H F_1 hybrid serving as a concurent control. No data are yet available.

c) The specific locus response of 101/H mice to specific locus mutation following spermatogonial X-irradiation is being studied. Informative amounts of data are not yet available.

3. Other strain differences:

a) Six inbred strains have been screened for differences in sensitivity to X-ray-induced spermatogonial stem celling killing. Recovered testis weight and duration of sterile period were the indicators used and the radiation dose was 6 Gy. Large differences

in sensitivity were observed. Sensitivity was highest with JU/Ct and its sub-strain JU/Ct-+^{a}+^{c}. Less sensitive were 129H and CBA/H and least sensitive were C3H/HeH and C57BL/6J. The 101/H strain studied previously, and considered highly sensitive, fitted into the intermediate category (129/H, CBA/H) of this range of strains. All of the strains were more sensitive than the C3H/HeH x 101/H hybrid.

b) The sensitivity of C3H/HeH stem cells to killing has been further investigated over a range of doses (1-7 Gy) to facilitate comparison with that of the 101/H strain and the hybrid. At all doses killing was lower than with the 101/H strain; at 1-2 Gy killing approximated that with the hybrid but at higher doses the C3H/HeH strain showed a greater sensitivity. The hybrid is therefore more resistant than either of its parent strains. The plateau in the C3H/HeH dose response curve appeared to be intermediate between that of 101/H mice (3-5 Gy) and that of the hybrid (6-8 Gy).

4. Cell stage sensitivity differences:

a) The translocation response of spermatogonial stem cells to those combined chemical-X-ray treatments that have recently found to enhance, or reduce, the specific locus mutation response to X-rays is being investigated. No data are yet available.

b) Specific locus mutation experiments to investigate this differing specific locus and translocation responses to fractionated X-ray doses have been initiated. Informative amounts of data are not yet available.

IV. Ojectives for the next reporting period:

1. To finalise all studies described above.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

CATTANACH, B.M., RASBERRY, C. and BEECHEY, C. A new steel allele with pre-implantation homozygous lethality. Mouse News Letter, 80, 156-157 (1988).

CATTANACH, B.M. and RASBERRY, C. A new steel allele with early post-implantation homozygous lethality. Mouse News Letter, 80, 157-158 (1988).

RASBERRY, C. and CATTANACH, B.M. Small ear, a new dominant mutation. Mouse News Letter, 80, 158-159 (1988).

EVANS, E.P., BURTENSHAW, M.D. and CATTANACH, B.M. A large deletion at the Sl locus. Mouse News Letter, 81: 66 (1988).

JONES, J., PETERS, J., BALL, S., CATTANACH, B.M. and KWON, B.S. A viable deletion at albino. Mouse News Letter In Press.

CATTANACH, B.M., PETERS, J. and RASBERRY, C. Induction of specific locus mutations in mouse spermatogonial stem cells by combined chemical-X-ray treatments. Mutation Research, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre National de la
Recherche Scientifique
15, Quai A. France
F - 75700 Paris

Contract no.: BI6-E-145-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. R. Devoret
Section de Radiobiologie Cellulaire
Lab. d'Enzymologie du C.N.R.S.
F - 91198 Gif-sur-Yvette

Telephone number: 69-82.34.72

Title of the research contract:

Induction of SOS functions from prokaryotes to higher eukaryotes.

List of projects:

1. Induction of SOS functions from prokaryotes to higher eukaryotes.

Title of the project no. 1:

Mechanisms of Induction of SOS Functions in Procaryotes and Eucaryotes.

Head of Project:

Raymond DEVORET

Scientific Staff:

R. Devoret, A. Bailone, P. L. Moreau, S. Sommer, M. Dutreix, J. Angulo, J. Célérier, A.-M. Dri.

I. Objectives of the project:

A) Since we demonstrated that a high cellular level of RecA protein protects chromosomal DNA against damage by physical and chemical carcinogens, we want to characterize the regulation of the RecA protein activities that determine such cellular processes as: (i) mutagenesis of the carcinogen-damaged cell, (ii) induction of recombinational repair, and (iii) amplification of RecA protein itself that is responsible for its protective effect. We aim at determining the nature of the SOS signal which triggers the induction of the coprotease activity of RecA protein.

B) Since we found a natural inhibitor of RecA protein activities, we want to characterize its action at the molecular level and assess the importance of its biological role in ensuring a homeostatic regulatory mechanism controlling activation versus inhibition of SOS functions.

C) We plan to identify proteins in lower eucaryotic cells and mammalian cells with activities similar to RecA protein. Such proteins may have a key role in mammalian SOS functions.

II. Objectives for the reporting period:

Our objectives were:

1) to isolate new recA mutations that, unlike classical pleiotropic mutations, would produce only one functional deficiency. We wanted to relate a given RecA functional deficiency with a specific change in the protein in the hope to characterize proteins domains.

2) to determine the mechanism whereby PsiB polypeptide counteracts the activities of RecA protein.

3) to identify a functional analog of RecA protein in mammalian cells.

III. Progress achieved:

New recA mutants with a dissociated phenotype. A third function of RecA protein is involved in mutagenesis.
(Dutreix, Bailone, Moreau, Galibert, Battista, Walker and Devoret)

1. Methodology

In order to locate the diverse enzymatic activities of RecA protein that determine the coprotease and recombinase functions, we set to isolate new recA mutants with a specific deficiency in protein cleavage. The recA gene of Escherichia coli was cloned into miniF plasmid. The advantage of using miniF plasmid as a vector is that the recA gene dosage being about one per chromosome, miniF plasmid produces a physiological cellular level of RecA protein. We isolated the new recA mutations by localized mutagenesis, their position was determined by sequencing.

2. Results

We obtained a few recA mutants displaying a distinct restrictive phenotype, each mutant being able to support cleavage of only one repressor. It is worth noting that one mutation, recA1730, prevents mutagenesis to occur even under conditions where SOS genes are derepressed and UmuD protein is cleaved. This property is found also in cells devoid of LexA repressor along with a relative cellular radiation resistance and an increased ability to recombine. The absence of mutagenesis is specifically linked to the recA1730 point mutation itself.

3. Discussion

We have provided evidence that, apart from the derepression of SOS genes and the cleavage of protein UmuD, there is a third specific requirement for RecA protein in mutagenesis produced by radiations. The RecA protein is mutagenic in that it helps the replisome to bypass the lesion inserting an A instead of the specific base. Two mechanisms can account for RecA action: (i) either RecA protein modifies some protein which is part of the replisome (ii) or RecA protein may cleave a third protein different from LexA and UmuD which allow lesion bypass.

A proposed mechanism for the activation of RecA protein in vivo.
(Moreau)

1. Methodology

It has been established that RecA protein must be activated to act as a coprotease in the cleavage of repressors such as LexA protein or the cI protein of a lambdoid phage. Activation of RecA protein is primarily controlled by single-stranded DNA, dNTPs and the presence of other proteins such as the single-strand-DNA binding protein (SSB).

2. Results

The level of SSB protein appears to influence the conversion of RecA protein from an initial coprotease activity to a subsequent recombinase activity. The latter activity stems from RecA property to polymerize on single-stranded DNA. Moreover, RecF protein may facilitate the conversion of RecA coprotease function into a recombinase by antagonizing SSB protein bound to single-stranded DNA.

3. Discussion

RecA protein activation appears to be controlled by a few proteins such as SSB and possibly RecF as shown also by other authors. The newly-found PsiB protein that antagonizes the activation of RecA protein may shed some light on the mechanism that ensures the switch between the two main functions of RecA protein to act as a coprotease or as a recombinase.

Plasmid PsiB protein prevents activation of RecA protein. Prevalence of PsiB gene among conjugative plasmids.

(Bailone, Bagdasarian, Bagdasarian, Sommer, Célérier and Devoret)

1. Methodology

There is an apparent biologial paradox: transfer of DNA during bacterial mating does not induce an SOS signal in the recipient cell in spite of the fact that plasmid DNA is transferred as single-stranded DNA. The presence of single-stranded DNA had been shown previously to generate an SOS signal. We looked for the existence of a function that would quench the formation of an SOS signal during conjugation. In other words, we looked for an inhibitor of SOS induction carried by a conjugative plasmid.

2. Results

We found that half of the naturally-occurring conjugative plasmids carry psiB, a gene that specifies an SOS inhibitor. Indeed, the PsiB protein can inhibit the induction of sfiA and prophage lambda. The plasmid psiB gene is situated near the origin of transfer of the plasmid DNA and this location may not be fortuitous. Most notable, we have demonstrated that PsiB protein inhibits the formation of an SOS signal by the activation of RecA protein. PsiB protein sensitizes the cell to radiations since the cells become phenotypically recA$^-$.

3. Discussion

The mechanism of inhibition of the formation of an SOS signal by PsiB protein may be caused by one of the two following mechanisms: PsiB protein may bind to RecA protein, or may bind to another protein needed for RecA protein activation. In any case, PsiB protein provides a new tool for the study of the mechanism of RecA protein activation. In particular, the isolation of recA mutants resistant to PsiB protein may permit to distinguish between the two just discussed mechanisms of inhibition of RecA activation.

KIN protein, an analog of RecA protein in mammalian cells?
(Angulo, Moreau, Bertolotti, Maunoury, and Devoret)

1. Methodology

We used antibodies raised against RecA protein of E. coli to identify a possible immunological analog of RecA protein in rat 3T3 cells.

2. Results

We have found a protein, called KIN (120 kDa), that is inducible by UV light and mitomycin C. KIN protein is located in the cell nucleus and is highly produced in actively dividing cells in mouse embryos.

3. Discussion

A protein of the same molecular weight has just been found by Drs. Fishel (NIH) and Rich (MIT). Our preliminary data show that the two proteins may be different.

IV. Objectives for the next reporting period:

1. Characterization of KIN protein in various mammalian cells.

We want first to clone the kin gene of rat cells. When the clone is obtained we will amplify and purify the KIN protein and then characterize the protein functions. We will also study the homology of KIN protein in various mammalian cells and possibly obtain the chromosomal location of the kin gene.

2. Characterization of the actions of PsiB and PsiA proteins.

We plan to characterize further how PsiB polypeptide prevents activation of RecA protein. Moreover, as we found that gene psiB is flanked by the psiA gene located downstream, we plan to investigate the biological role of PsiA protein and show whether its interacts with PsiB protein.

3. Correspondence between recA mutations, RecA protein functions and the domains of the protein in repressor cleavage and mutagenesis. The changes in phenotypes of the new recA mutants obtained will be related to the physical location of the mutations to propose a possible model for the structure of RecA protein.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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Dr. C. Jacq, Ecole Normale Sup., 75005 PARIS.
Dr. C. M. Radding, Yale Medical School, NEW HAVEN, CT 06510.

VI. Publications:

1988

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Dutreix M, Bailone A, Devoret R (1985) Efficiency of induction of prophage lambda mutants as a function of recA alleles. J Bacteriol 161:1080-1085

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RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-147-F

Institut Curie
Section Biologie
Rue d'Ulm, 26
F - 75231 Paris Cédex 05

Head(s) of research team(s) [name(s) and address(es)]:

Dr. B. Dutrillaux
Section Biologie
Institut Curie
Rue d'Ulm, 26
F - 75231 Paris Cédex 05

Telephone number: 1-43.29.12.42 (Ext. 3350)

Title of the research contract:

Somatic cytogenetics of normal humans and people suspected of having a defect in the repair of DNA damage.

List of projects:

1. Study of the chromosomal constitution of normal humans and the effect of low doses of radiation.
2. Cytogenetic study of abnormal genomes particularly in cells carrying a suspected error in the repair of DNA.

Title of the project no.:

Study of chromosomal constitution of normal humans and effect of low doses of radiation.

Head(s) of project:

B. DUTRILLAUX

Scientific staff:

ALEDO-ZAMORA R.	LEFRANCOIS D.	VIEGAS-PEQUIGNOT E.
AURIAS A.	MAMURIS Z.	
COUTURIER J.	MULERIS M.	
DUTRILLAUX B.	PRIEUR M.	

I. Objectives of the project:

Better knowledge of the so-called spontaneous chromosomal anomalies occurring in human lymphocytes and study of the effect of radiations at low doses. Relationship with aging and various pathological conditions.

II. Objectives for the reporting period:

Identification of chromosomal rearrangements induced by low doses of radiations. Study in new-borns. Study of lesions induced by an alkylating agent and comparison with radiation induced lesions.

III. Progress achieved:

Methodology

Human lymphocyte cultures were performed during 48, 72 and 96 h. Exposure to radiation (γ-rays, 0, 0.05, 0.10, 0.20 and 0.50 Gy) at Go-phase, exposure to melphalan at G1 phase. Each metaphase was analysed after photography by 2 independent observers. Chromosome staining = R-banding.
Cell cycle analysis by BrdU (5-bromodeoxyuridine) and FPG staining.

Results and discussion

In the previous reports, we established the frequencies and the types of spontaneous occurring chromosomal rearrangements in lymphocytes from controls of various ages. It was shown that the frequency of some rearrangements was not age dependent whereas others were. Using this information, the effect of low doses radiations was studied.

In lymphocytes from young adults chromosomal aberrations were not detected at doses of .05 and .1 Gy, and in lymphocytes from old adults not even at .2 Gy. The difficulty in detecting aberrations in lymphocytes from adults is largely due to a considerable background of chromosomal anomalies which should be borne in mind in dosimetry studies. The rate of induction largely depends on the types of rearrangements. One-break terminal deletions are efficiently induced at .1 and .2 Gy and are the best indicators of exposure at these doses. At .5 Gy mainly dicentrics were found.

Finally, it became clear that the effect of low doses (around .05 Gy) can be estimated in new-borns only.

This is the reason why a study of samples from new-borns is now in progress.

Studies of lymphocytes exposed to melphalan, "in vivo" and "in vitro" gave the following results.

The "in vitro" results showed that after 1, 2 and 3 cell cycles, unbalanced aberrations are quite frequent and balanced aberrations rare. Chromosomes 5, 7, 11 and 17 are frequently affected. R-bands are significantly more affected than G-bands. The frequent breakage of these chromosomes and of R-bands which are known to be G-C rich, may result from the preferential methylation of guanine by melphalan. The analysis of PHA stimulated lymphocytes from 24 patients previously treated with alkylating agents with or without s-ANLL showed that chromosomal aberrations are quite frequent, affecting 21,5 % of metaphases on the average. Reciprocal translocations are more frequent than unbalanced aberrations. Chromosomes 5, 7, 11 and 17 are also overinvolved in aberrations. These 4 chromosomes are also overinvolved in clonal aberrations of the s-ANLL. It is likely that these treatments induced non random mutations of recessive genes located on these chromosomes by a targeted mutagenesis. Then various aberrations including deletions of the homologous normal counterparts may occur, unmasking the mutated recessive genes. This stage would be concomitant with the leukemic progression.

IV. Objectives for the next reporting period:

- Continuation of the study of aberrations induced by melphalan.
- Continuation of the study of the effect of low doses of radiations on the lymphocytes from new-borns.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Announced in the previous report

1. Prieur M., Al Achkar W., Aurias A., Couturier J., Dutrillaux A.M., Dutrillaux B., Flüry-Hérard A., Gerbault-Seureau M., Hoffschir F., Lamoliatte E., Lefrançois D., Lombard M., Muleris M., Ricoul M., Sabatier L. and Viegas-Péquignot E.
Acquired chromosome rearrangements in human lymphocytes : effect of aging. Hum. Genet., 79 : 147-150 (1988).

Other publications

2. Lefrançois D., Al Achkar W., Aurias A., Couturier J., Dutrillaux A.M., Dutrillaux B., Flüry-Hérard A., Gerbault-Seureau M., Hoffschir F., Lamoliatte E., Lombard M., Muleris M., Prieur M., Ricoul M., Sabatier L. and Viegas-Péquignot E.
Chromosomal aberrations induced by low dose -irradiation. Study of R-banded chromosomes of human lymphocytes. Mutation Res. (in press).

3. Mamuris Z., Gerbault-Seureau M., Prieur M., Pouillart P., Dutrillaux B. and Aurias A.
Chromosomal aberrations in lymphocytes of patients treated with melphalan. Int. J. Cancer, (in press).

Title of the project no.:

Cytogenetic study of abnormal genomes particularly in cells carrying a suspected error in the repair of DNA.

Head(s) of project:

Alain AURIAS

Scientific staff:
ALEDO-ZAMORA R.
DUTRILLAUX B.
STERN M-H.
ZHANG F.

I. Objectives of the project:

Better knowledge of the chromosomal anomalies in genetic diseases which increase the risk of cancer. Improvement of the methods of diagnosis of the homozygote, and possibly heterozygote status.

II. Objectives for the reporting period:

Study of the karyotypes of patients affected by Ataxia telangiectasia, Fanconi anaemia, Xeroderma pigmentosum and Retinoblastoma.
Research of chromosomal aberrations involved in clonal cells and characterization of the breakpoints observed in these aberrations.
In situ hybridization of probes for immunoglobulin and related genes on metaphases carrying clonal rearrangements. Chromosome study in skin cancers from xeroderma pigmentosum patients.

III. Progress achieved:

Methodology

The analysis of patients affected by precancerous diseases was mainly focused on xeroderma pigmentosum. Blood, skin, and tumor cell cultures were developed. The study of unscheduled DNA synthesis (UDS) was performed. Sister chromatid exchanges and chromosome lesions were quantified. In situ hybridization on chromosomes was applied to characterize breakpoints in ataxia telangiectasia.

Results and discussion

- Xeroderma pigmentosum :

1) Chromosome breaks:

The study performed on 978 R-banded metaphases shows that there is no specific chromosomal rearrangement in this disorder. In UDS-deficient forms, the rates of deletions, chromatid gaps and chromosome gaps are significantly increased. A preferential involvement of G-bands is found.

2) Sister chromatid exchanges:

The distribution of spontaneous sister chromatid exchanges (SCEs) was studied in PHA-stimulated lymphocytes from 15 patients affected by xeroderma pigmentosum (XP). The study of UDS in twelve of these patients showed that seven were deficient and five proficient. The number of SCEs in XP patient cells was higher than in those of 19 controls, and the distributions of SCEs per cell were significantly different. However, the results varied when XP patients were considered in relation to their UDS: the group of XP patients with proficient UDS did not differ, whereas the group of XP patients with deficient UDS was very significantly different from controls. The group not tested for UDS was similar to the deficient UDS group. The possible relationship between the increase of SCEs and the type of DNA repair defect is discussed.

3) Tumor cells:

The cytogenetic study of a case of cutaneous squamous cell carcinoma developed in a child affected by xeroderma pigmentosum is described. In this paratetraploid tumor, virtually all mitoses had the following rearrangements: i(1q), i(1p), t(3q14q), del(9p), and der(19)t(8;19). In addition, there were several deletions of 1p and 1q. The del(9p) likely occurred as the first rearrangement. The distal segment of the short arm of chromosome # 9 and the long arm of # 19 and # 22 were the most underrepresented and chromosome # 6 the most overrepresented chromosome or chromosome segment. The most striking anomaly detected was a jumping translocation of chromosome # 14, involved with chromosomes # 1, # 3, # 5, # 7, # 9, # 14, and # 22. The breakage of chromosome # 14 always occurred on the short arm.

- Ataxia telangiectasia :

To delimit the 14q32.1 recurrent breakpoint of ataxia telangiectasia clones, we performed an in situ hybridization study with various probes located on the 14q32 band. We thus mapped this breakpoint between the D14S1 and Pi loci. Furthermore, an interstitial duplication including D14S1 and a part of the IgH locus was demonstrated on a t(14;14) clone.

IV. Objectives for the next reporting period:

- Continuation of the study of patients.
- Study of the clones at the chromosomal and molecular level.
- Correlated study of chromosomal anomalies experimentally induced in vitro and in vivo, and in patients with secondary leukemias, following radiation or chemotherapy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Pr. C. GRISCELLI	Hôpital Enfants Malades, Paris
Pr. R. GATTI	UCLA, Los Angeles
Dr. I. KIRSCH	NCI, Bethesda
Dr. M.F. AVRIL	Institut Gustave Roussy, Paris (Villejuif)
Dr. A. SARASIN	Institut de Recherche Scientifique sur le Cancer, Paris (Villejuif).

VI. Publications:

Announced in the previous report

1. Zhang F., Stern M.H., Thomas G. and Aurias A. Molecular characterization of ataxia telangiectasia T cell clones. II. The clonal inv(14) in ataxia telangiectasia differs from the inv(14) in T cell lymphoma. Hum. Genet., 78 : 316-319 (1988).
2. Aledo R., Aurias A., Chrétien B. and Dutrillaux B. Jumping translocation of chromosome 14 in a skin squamous cell carcinoma from a xeroderma pigmentosum patient. Cancer Genet. Cytogenet., 33 : 29-33 (1988).

Other publications

3. Aledo R., Avril M.F., Dutrillaux B. and Aurias A. Spontaneous chromosomal anomalies in lymphocytes from xeroderma pigmentosum. A study of ten patients. Ann. Génét., 31 : 211-215 (1988).
4. Aledo R., Renault G., Prieur M., Avril M.F., Chrétien B., Dutrillaux B. and Aurias A. Increase of sister chromatid exchanges in excision repair deficient xeroderma pigmentosum. Hum. Genet., (in press).
5. Stern M.H., Zhang F., Thomas G., Griscelli C. and Aurias A. Molecular characterization of ataxia telangiectasia T cell clones.III.Mapping the 14q32.1 distal breakpoint. Hum. Genet., 81 : 18-22 (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Commissariat à l'Energie Atomique
Institut de Protection et de
Sûreté Nucléaire
B.P. n° 6
F - 92265 Fontenay-aux-Roses

Contract no.: BI6-E-149-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. B. Dutrillaux
Laboratoire de Génét. Expérimentale
CEA-IPSN
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F - 92265 Fontenay-aux-Roses

Telephone number: 01-46.54.83.27

Title of the research contract:

A qualitative study of radiation-induced chromosomal breakage and development of a test for radiation sensitivity.

List of projects:

1. A qualitative study of radiation-induced chromosomal breakage and development of a test for radiation sensitivity.

Title of the project no.:

Qualitative study of radiation-induced chromosomal breakage and development of a test for radiation sensitivity.

Head(s) of project:

B. DUTRILLAUX

Scientific staff:

AL ACHKAR W.
HOFFSCHIR F.
SABATIER L.

I. Objectives of the project:

1. Comparison of the types of chromosomal rearrangements induced by γ-rays and by heavy ions.
2. Comparison of the types of chromosomal rearrangements induced in various mammals, including man, selected for their karyotypic pecularities.
3. Assessment of the transmission of various chromosomal rearrangements through cell division.

II. Objectives for the reporting period:

- Study of the effect of caffeine on radiation sensitivity in normal cells and in cells from patients affected by Fanconi anemia.
- Comparison of chromosome lesions induced by various type of radiations.
- Study of the transmission of radiation induced rearrangements through cell divisions.
- Influence of time and cell cycle on radiation induced chromosome lesions.

III. Progress achieved:

Methodology

All experiments were performed on PHA stimulated lymphocytes. Incorporation of 5-bromodeoxyuridine were applied to study cell kinetics, in relation with R-banding to study chromosomal rearrangements. Treatments by caffeine were developed.

Results and discussion

Effect of caffeine :

In Fanconi anemia (FA) cells the duration of the G_2 phase of the cell cycle prolonged. Such a slowing of the G_2 phase can be induced in normal cells by irradiation with γ rays during S phase, which also further increases the duration of G_2 in FA cells. The addition of caffeine during the last 7 h of culture shortens the G_2 phase in both nonirradiated and irradiated FA cells. In nonirradiated normal cells it may have no effect or may increase G_2 phase duration, but in irradiated normal reduces the slowing of G_2 induced by the radiation. This suggests that FA cells recognize and repair preexisting DNA lesions during G_2 phase and that caffeine inhibits this process. The principal anomaly in FA may be a deficient repair during S phase, as manifest in the prolonged postreplication repair period during G_2 phase required to repair the larger number of lesions passing through S phase.

Influence of time and cell cycle phase on radiation-induced chromosome lesions :

Irradiation of human lymphocytes with γ -rays were performed every hour from 7 to 1 h before harvesting and BrdU was added to the cultures immediatly after irradiation to estimate the cell cycle phase by studying replication band patterns for each analysed metaphase. The average number of lesions has been observed to be more related to the time elapsed between irradiation and harvesting than to the cell phase. However, the types of lesions i.e. chromatid, chromosome breaks and chromatid exchanges are closely dependent on the phase. Cells irradiated 2 h before harvesting exhibit 3 and 6 times more lesions than those irradiated 3 and 7 h before harvesting respectively. This high sensitivity of cells, 2 h before harvesting, is observed for cells irradiated during late S as well as during G_2 phase.

IV. Objectives for the next reporting period:

- Continuation of the work on heavy ions.
- Characterization of "hot spots" and "cold spots" of rearrangement of human chromosomes after irradiation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Announced in the previous report

1. Hoffschir F., Prieur M. and Dutrillaux B.
Diagrammatic representation for chromosomal mutagenesis studies IV. Radiation-induced rearrangements in Ateles sp. (Primate, Platyrhini). Mutation Res., 199 : 103-110 (1988).

2. Al Achkar W., Sabatier L. and Dutrillaux B.
Transmission of radiation-induced rearrangements through cell divisions. Mutation Res., 198 : 191-198 (1988).

Other publications

3. Al Achkar W., Sabatier L. and Dutrillaux B.
Influence of time and cell cycle phase on radiation-induced chromosome lesions. Ann. Génét., 31 : 87-90 (1988).

4. Sabatier L. and Dutrillaux B.
Effect of caffeine in Fanconi anemia. I. Restoration of a normal duration of G_2 phase. Hum. Genet., 79 : 242-244 (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-156-D

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
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Head(s) of research team(s) [name(s) and address(es)]:

Dr. U.H. Ehling
Institut für Genetik
GSF
Ingolstädter Landstrasse 1
D - 8042 Neuherberg

Telephone number: 089-31872346

Title of the research contract:

Radiation-induced mutations in mammals.

List of projects:

1. Radiation-induced dominant cataract mutations in mammals.
2. Biochemical analysis of cataract mutants in mice.
3. Radiation-induced gene mutations causing alterations of enzymes.

Title of the project no.: 1

Radiation-induced dominant cataract mutations in mammals

Head(s) of project:

J. Favor

Scientific staff:

U.H. Ehling
J. Kratochvilova

I. Objectives of the project:

a) Species comparison of the radiation-induced mutation rate to dominant cataract alleles.

b) Dose response analysis of the radiation-induced mutation rate to dominant cataract alleles in the mouse.

c) Estimation of the radiation-induced mutation rate to dominant cataract and recessive specific locus alleles in treated female mice.

d) Phenogenetic and biochemical characterization of radiation-induced mutations.

II. Objectives for the reporting period:

a) The species comparison between goloen hamster and the mouse for the sensitivity to 2+2 Gy radiation-induced mutation rate to dominant cataract alleles will be continued.

b) An extended control group for dominant cataract mutation rate experiments in the mouse will be completed.

c) A 3 Gy oocyte irradiation experiment will be initiated.

d) Recovered dominant cataract mutations will be phenotypically and genetically characterized.

III. Progress achieved:

Procedures

The control group, for which the spontaneous mutation rate to recessive specific locus and dominant cataract mutation rates were estimated, has been extended. Homozygous wildtype (102/ElxC3H/El)F_1 hybrid male mice were mated to female Tester-stock mice, homozygous recessive at seven specific loci controlling hair pigmentation and the size of the external ear (a, b, c, d, p, se, s). Offspring were screened for phenotypic variants indicative of recessive specific locus or dominant cataract mutations. Presumed specific locus mutations were genetically confirmed by a test of allelism at the suspected locus, while the genetic nature of presumed cataract mutations was comfirmed by transmission in an outcross.

The doubling dose for radiation-induced specific locus and dominant cataract mutations was estimated, based on single, acute dose-rate (0.75 Gy/min), spermatogonial γ-irradiation of the mouse. The doubling dose is defined as that dose which induces as many mutations as occur spontaneously per generation. It is calculated as the ratio between the spontaneous mutation rate and the induced mutation rate per unit dose.

Results and Discussion

The number of recovered specific locus and dominant cataract mutations as well as the number of offspring examined for the control and irradiated groups are given in the Table. The present results have

Dose (Gy)	Specific Locus Mutation	Offspring	MR*	DD**	Dominant Cataract Mutation	Offspring	MR*	DD**
0								
Previous control	19	227 805	1.19		1	22 594	0.15	-
Present control	3	20 878	2.05		3	20 629	0.48	-
Total	22	248 683	1.26		4	43 223	0.31	-
1.5	11	28 964	5.43	0.45	2	23 157	0.29	-
3.0	7	24 416	4.10	1.33	3	22 712	0.44	7.15
5.3	7	10 212	9.79	0.78	3	10 212	0.98	2.45
6.0	15	18 176	11.79	0.72	3	17 599	0.57	7.15
6.0	14	11 095	18.03	0.45	3	11 095	0.90	3.15

* Mutations/locus/gamete x 10^{-5}; for specific locus mutations 7 loci were screened; for dominant cataract mutations 30 loci are assumed.
** Doubling dose (Gy)

doubled the size of the dominant cataract control group, with an observed mutation frequency of 4/43 223. The doubling dose estimates are presented

for each experimental dose point. For the specific locus results, doubling dose estimates ranged from 0.45 Gy to 1.33 Gy, with a weighted mean of 0.77 Gy. This is higher than the previously published estimate of the doubling dose for acute spermatogonial irradiation in the mouse, 0.34 Gy (PNAS, USA 79: 542-544, 1982). The reason for this discrepancy may be two-fold: either there is a difference in the observed spontaneous mutation rates, or there is a difference in the effectiveness of irradiation in inducing specific locus mutations. The source of this discrepancy should be pursued since the radiation doubling dose for induced mutations estimated from mouse specific locus experiments forms the basis for a genetic risk estimation of man.

The estimated doubling doses for radiation-induced dominant cataract mutations were higher than the specific locus doubling doses. Estimates ranged from 2.45 Gy to 7.15 Gy (the negative estimate at 1.5 Gy has been assigned a value of 0) with a weighted mean of 4.13 Gy. These observations confirm previous preliminary results which indicated a higher radiation doubling dose for induced dominant cataract mutations then for induced recessive specific locus mutations. This more than five-fold difference in the estimated doubling dose for radiation-induced dominant cataract mutations when compared to the doubling dose for specific locus mutations violates a basic assumption required for a genetic risk estimate in man based on mouse specific locus data employing the indirect estimation procedure; i.e. that the doubling dose estimated in the mouse for the recessive specific locus test is representative for all genetic endpoints. However, it should be noted that the specific locus data are inherently less subject to experimental variability since 1) the historical control is more than five times larger and 2) the expected number of mutations observed at each dose point is greater. It is of critical importance to make a valid comparison of the estimated radiation doubling dose based on specific locus and dominant cataract mutation rate data. Therefore, any shortcomings of the dominant cataract data should be rectified. We propose as an initial step to increase the control sample size.

IV. Objectives for the next reporting period:

a) Extend the control sample size for dominant cataract mutations in the mouse.
b) The strain comparison of sensitivity to radiation-induced specific locus and dominant cataract mutations will be continued for inbred strain AKR.
c) An estimate of the induced mutation rate in oocytes of the mouse following 3 Gy irradiation will be continued.
d) The species comparison between golden hamster and the mouse for the sensitivity to radiation-induced dominant cataract mutations will be continued at 2+2 Gy.
e) Recovered dominant cataract mutations will be phenotypically and genetically characterized.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Ehling, U.H., and A. Neuhäuser-Klaus: Induction of specific-locus and dominant-lethal mutations by cyclophosphamide and combined cyclophosphamide-radiation treatment in male mice. Mutation Research 199, 21-30, 1988

Ehling, U.H.: Methods to estimate the genetic risk. Genome, in press.

Ehling, U.H.: Germ cell mutations in mice. Proceedings "Perinatal and Multigeneration Carcinogenesis", Leningrad, 31.5.-2.6.1988, in press.

Ehling, U.H.: Induction of mutations in mice: Standards for protecting the human genome. Revista Latinamericana de Genética, in press.

Ehling, U.H.: EEMS-Award Lecture. Mutation Research and Biologisches Zentralblatt, in press.

Favor, J.: Risk estimation based on germ cell mutations in animals. Genome, in press.

Favor, J.: Mammalian germ cell mutagenesis data and human genetic risk. Biologisches Zentralblatt, in press.

Kratochvilova, J., and J. Favor: Phenotypic characteriziation and genetic analysis of twenty dominant cataract mutations detected in offspring of irradiated male mice. Genet. Res., Camb. 52, 125-134, 1988.

Title of the project no.: 2

Biochemical analysis of cataract mutants in mice

Head(s) of project:

J. Graw

Scientific staff:
A. Liebstein
T. Werner

I. Objectives of the project:

a) The lenses of 10 dominant cataract mutants recovered in radiation experiments of mice will be analyzed systematically with biochemical methods (including protein analysis, determination of metabolite concentrations and enzyme activities).

b) The Nop-cataract of the mouse is a nuclear opacity, which is inherited as an autosomal dominant gene. Among the proteins of the lens, gamma-crystallins were diminished as compared to the wild type. The cause for the reduced amount of these proteins will be investigated.

II. Objectives for the reporting period:

a) Biochemical analysis of cataractous lenses from mutants recovered in radiation experiments.

b) Sequence analysis of gamma-crystallin specific DNA probes derived from murine genomic DNA libraries (wild type and homozygous cataract mutant Nop).

III. Progress achieved:

Procedures

The mutants were investigated with respect to the lenticular protein pattern as analyzed by isoelectric focusing on polyacrylamide gels (PAGIF) or by polyacrylamide gel electrophoresis (PAGE). Gamma-crystallin specific DNA clones from genomic libraries (wild type or Nop/Nop) were characterized by restriction analysis. For sequence analysis, interesting parts of the DNA were subcloned in pUC19 and analyzed by gel electrophoresis using the dideoxy chain termination method.

Results and Discussion

a) The biochemical analysis of cataractous lenses was performed in order to determine which metabolic pathways are affected in the cataractous lenses to describe the pathogenesis. The investigations revealed a great number of variations between the wild types and the different mutants with respect to lenticular enzyme activities and metabolite concentrations. However, this approach does not lead to the detection of the primary defects in the mutants; most of the observed changes likely reflect secondary events. Therefore, this particular approach was not further pursued. Instead parameters were chosen for study which reflect lens differentiation. Few parameters (e.g. crystallins, cytoskeletal proteins) might be used as biochemical indicators for lens developmental alterations. The biochemical analysis of two cataract mutants is presented:

Cat-2^t mouse with total cataract. The mutation is radiation-induced (2 x 5.1 Gy, 24 hrs interval), autosomal dominant and without effects on viability and fertility (formerly called R-324 [Graw, J., Favor, J., Neuhäuser-Klaus, A., Ehling U.H., Mutation Res., 159, 1986, 47-54]);

Nop mouse with nuclear opacity of the lens. The mutation is of spontaneous origin, autosomal dominant and without effects on viability and fertility (Graw, J., Kratochvilova, J., Summer, K.-H., Exp. Eye Res., 39, 1984, 37-45).

Both murine cataract mutants exhibit altered protein pattern in the PAGIF. Proteins in the basic region were diminished; they can be identified to be gamma-crystallins, one of the structural lens proteins. Additionally, both murine cataract mutants exhibit an altered pattern of water soluble lens proteins at molecular weights above 35 kd. In the Nop mouse, this alteration can be observed only in the homozygotes. Western

blot analysis in the Cat-2^t mouse revealed that some of the bands at higher molecular weight are the cytoskeletal proteins actin and vimentin. Such proteins usually can be detected in the urea soluble fraction of the lens. Vimentin and gamma-crystallins can be employed to follow lenticular differentiation from epithelial to fiber cells: gamma-crystallins are predominantly present in fiber cells, but not in epithelial cells. Vimentin, on the other hand, has been found primarily in the epithelial and cortical fiber cells; it is largly absent from the lens nucleus. Therefore, it is concluded that cataractogenesis in both mutants presented here is accompanied by alterations in lenticular differentiation.

b) The genomic organization of gamma-crystallins in mice was analyzed. From the genomic library of wild-type mice five different clones were identified to be gamma-crystallin specific. However, one clone was shown by restriction site mapping to be different from any of the described murine gamma-crystallin genes. The difference to the described murine gamma-crystallin genes was confirmed by sequence analysis. Although some regulatory elements, the first exon and parts of the second exon are highly homologous to the other murine gamma-crystallin genes, the noncoding regions are very different. Preliminary analysis of the sequence lead to its classification as a gamma-E-crystallin, a member of the gamma-crystallin gene family, which has not yet been described in mice (for review see Aarts, H.J.M., den Dunnen, J.T., Leunissen, J., Lubsen, N.H., Schoenmakers, J.G.G., J. Mol. Evol. 27, 1988, 163-172). The three genomic clones obtained in the DNA-library from the homozygous Nop mutants were further characterized by restriction mapping. Although the clones hybridize to an oligonucleotide from the highly conserved part of the 5' region and to a gamma-crystallin specific cDNA, neither the restriction maps nor the hybridization with gamma-crystallin specific oligonucleotides for other non 5' regions indicated similarity to the already described gamma-crystallin genes. Therefore, a determination of the DNA sequence of the gamma-crystallin specific regions from the clones obtained in the Nop-library was initiated. Preliminary analysis of the few sequences studied indicated only weak homologies to the known gamma-crystallin genes.
DNA-binding studies using a 230 bp AvaII/AluI DNA fragment from the 5'

regulatory region in a gel retention assay exhibited an altered banding pattern comparing lens extracts from wild type and Nop mutants suggesting that the regulation of the gamma-crystallin transcription is affected. The main question, however, if an altered cis- or trans-regulation of gamma-crystallins may explain the cataractogenesis in the Nop-mutant, can be answered only when the sequence study is completed.

IV. Objectives for the next reporting period:

1. The sequence analysis of the gamma-crystallin genes in wild type and the Nop mutant will be continued focussed on the description of the 5' regulatory regions.
2. Experiments will be initiated to study the transcription of gamma-crystallin genes in the wild type as well as in the Nop mice.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Graw, J., P.M. Gopinath, W. Bors, C. Michel and H.K-H. Summer: Biochemical analysis of young rats homozygous for the cataract mutation cat. Exp. Eye Res. 48, 1-9, 1989.

Title of the project no.: 3

Radiation-induced gene mutations causing alterations of enzymes

Head(s) of project:

W. Pretsch

Scientific staff:

S. Merkle
A. Neuhäuser-Klaus

I. Objectives of the project:

To determine the mutation rates at loci controlling erythrocyte enzyme activity in offspring of irradiated mice, in a combined experiment scoring specific locus mutants in the same offspring. To characterize genetically and biochemically the recovered enzyme-activity mutants.

II. Objectives for the reporting period:

Offspring from 3+3 Gy (24-h fractionation interval) irradiated AKR males were examined for enzyme-activity mutations. The recovered mutants were genetically characterized.

III. Progress achieved:

Procedures

AKR male mice, homozygous a/a c/c, were exposed to 3+3 Gy X-irradiation (24-h fractionation interval; 0.43 Gy/min) and each male was mated with an untreated test-stock female, homozygous for 7 recessive loci (a, b, c^{ch}, d, p, s, se). Offspring were screened for recessive mutations at the 5 specific loci at which animals were heterozygous (b, d, p, s, se) and a sub-group was examined for alterations of erythrocyte enzyme activity for 10 enzymes controlled by 12 loci: lactate dehydrogenase (LDH), triose phosphate isomerase, malate dehydrogenase (MDH), glucose phosphate isomerase, phosphoglycerate kinase, phosphoglycerate mutase (PGAM), glyceraldehyde phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, pyruvate kinase, and glutathione reductase (GR). Individuals with specific activities (expressed as units/g haemoglobin) either above or below 3 standard deviations of the mean were considered as outliers. If this enzyme-activity alteration was confirmed in a second blood sample, the presumed mutant was outcrossed to the inbred strain C3H/El for genetic confirmation. Presumed specific locus mutations were confirmed by an allelism test at the suspected locus. The enzyme-activity mutations were maintained by backcrossing to inbred C3H/El mice. Heterozygous mutants were mated inter se for investigation of homozygous viability of the mutated gene. Litter size at weaning and transmission ratio were determined for the mutations.

Results and Discussion

Two enzyme-activity mutants were detected in 1 063 offspring from treated post-spermatogonial cell stages (pg) and 6 mutants were observed in 5 068 offspring descendent from irradiated spermatogonia (g). The per locus mutation rate of enzyme-activity mutations is comparable to that of specific locus mutations.

Treated germ cell stage[a]	Enzyme-activity (12 loci) mutants/ offspring	mutation rate[b]	Specific locus (5 loci) mutants/ offspring	mutation rate[b]
pg	2 / 1 063	15.7	1 / 1 115	17.9
g	6 / 5 608	8.9	6 / 7 635	15.7

[a] pg: post-spermatogonial cell stages; g: spermatogonia
[b] (mutations/locus/offspring)x10^{-5}

Two of the mutants have elevated enzyme activities (PGAM 46, PGAM 1042); the remaining six express decreased activities. The dominant mode of

inheritance of the altered enzyme activities in the mutants has been demonstrated by backcrossing (B) with C3H/El. The relative transmission was calculated as the percent of mutant offspring observed compared to that expected (50% in backcross). Transmission of the two mutants induced in post-spermatogonial cell stages (MDH 140, GR 419) is drastically reduced; however it must be considered that the tested number of offspring is relatively small. Transmission for the mutants recovered in treated spermatogonia is normal.

To determine homozygous viability of the mutations, mutant heterozygotes were crossed inter se (I) and offspring were classified for enzyme activity. Five of the 8 mutations have been tested and no animal with a more extreme phenotype than the heterozygotes was observed in offspring from intercrosses. Four of the five tested mutations also have a segregation ratio of approximately 1:2, which is consistent with homozygous lethality of the induced mutation. Preliminary results for the fifth tested mutation (LDH 2534) indicate a segregation ratio of 1:2.85 and do not exclude the possibility that the mutation is homozygous viable and completely dominant.

To date of 14 radiation-induced enzyme-activity mutants, 13 have been shown to be homozygous lethal. In contrast, out of 36 activity mutants originating from ethylnitrosourea experiments, only 19 mutants are homozygous lethal. This is consistent with the suggestion that radiation-induced mutations recovered in germ cells of mice are more likely deletions whereas ethylnitrosourea-induced mutations are more likely point mutations.

Mutant line	Wild-type activity (%)	Type of mating[a]	Litter size ($\bar{x} \pm$ SD)	Genotype[b] +/+	+/+*	+*/+*	Transmission (%)
MDH 140	50	B	4.3 ± 0.5	13	4	-	47
		I	not tested				
GR 419	50	B	5.0 ± 2.9	15	5	-	50
		I	not tested				
LDH 2534	50	B	8.6 ± 1.2	60	44	-	85
		I	6.8 ± 0.9	7	20	0	

PGAM 46	300	B	7.8 ± 2.1	69	71	-	101
		I	6.1 ± 1.0	11	20	0	
PGAM 737	70	B	8.2 ± 2.1	117	94	-	90
		I	not tested				
PGAM 1042	160	B	7.9 ± 1.0	83	76	-	96
		I	6.9 ± 1.0	56	65	0	
GR 205	50	B	5.4 ± 1.5	93	63	-	81
		I	4.6 ± 1.5	18	32	0	
GR 2364	50	B	7.6 ± 2.0	43	61	-	117
		I	7.5 ± 3.3	8	19	0	

[a] B: backcrosses (+/+)x(+/+*); I: intercrosses (+/+*)x(+/+*)
[b] +/+: wild types; +/+*: heterozygous mutants; +*/+*: homozygous mutants

IV. Objectives for the next reporting period:

- Some selected radiation-induced mutations with altered phosphoglycerate mutase or glutathione reductase activity will be characterized biochemically.
- The control sample size for enzyme-activity mutations in offspring from (101/ElxC3H/El)F_1 males will be extended.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Pretsch, W.: Eight independent Ldh-1 mutations of the mouse recovered in mutagenicity experiments: biochemical characteristics and chromosomal localization, Genetical Research, Camb., in press.

Favor, J., A. Neuhäuser-Klaus, J. Kratochvilova and W. Pretsch: Towards an understanding of the nature and fitness of induced mutations in germ cells of mice: homozygous viability and heterozygous fitness effects of induced specific locus, dominant cataract and enzyme activity mutations, Mutation Research, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-205-I

Università di Roma "La Sapienza"
Dipartimento di Biopatologia Umana
Piazzale Aldo Moro, 5
I - 00185 Roma

Head(s) of research team(s) [name(s) and address(es)]:

Prof. R. Elli
Facoltà di Medicina e Chirurgia
Università di Roma "La Sapienza"
Viale Regina Elena, 324
I - 00161 Roma

Telephone number: 06-49.00.47

Title of the research contract:

Response to radiations of human cells modified by pR plasmid that confers radioresistance in bacteria.

List of projects:

1. Response to radiations of human cells modified by pR plasmid that confers radioresistance in bacteria.

Title of the project no.:

RESPONSE TO RADIATIONS OF HUMAN CELLS MODIFIED BY pR PLASMID THAT CONFERS RADIORESISTANCE IN BACTERIA

Head(s) of project:

Prof. R.ELLI

Scientific staff:

F.Gigliani, L.Marcucci, A.Antonelli, P.Petrinelli, P.A.Battaglia, D.Kobal

I. Objectives of the project:

The increasing number of observed phenomena, including enhanced DNA repair, virus induction, induced cellular differentiation and neoplastic transformation, phenomena resulting from DNA damage or replication arrest, suggest that there is an SOS-like system in mammalian cells.The objective of this project is to ascertain in what measure this inducible system is correlated with mutagenesis and carcinogenesis.For this purpose it will be utilized the pR plasmid that interacting with the inducible repair pathway confers increased survival to UV and 4NQO in prokaryotic as in mammalian cells.This system has the following advantages: it permits the evaluation of inducible response to stress, directly in the damaged cells;it makes it possible to understand the molecular and genetic processes of the inducible response related to mutagenesis and carcinogenesis.

II. Objectives for the reporting period:

The experimental data obtained in recent years allowed us to conclude that pR works in a similar way both in mammalian and in bacterial cells and that it plays an important role in the mammalian DNA repair mechanisms that modulate the cytotoxic effect of DNA damaging agents.
The two main objectives of the study are:
1) to clarify the function of the uvp1 and uvp2 regions of pR plasmid and the interaction between them through the analysis of bleomycin response of bacterial and mammalian cells transformed with mutated pR plasmids;
2) to characterize the uvp1 region in terms of nucleotide sequence, codified protein and function in bacterial cells.

III. Progress achieved:

METHODOLOGY

- Southern blot hybridization was performed as described by E.N.Southern (J.Mol.Biol., 98, 503, 1975)
- Dot blot hybridization was performed as described by F.C.Kafatos et al. (Nucleic Acids Res., 7, 1541, 1979)
- Mini-cells technique was performed as described by Rambach and Hoghness (Proc.Natl.Acad.Sci., 74, 5041, 1977)
- Nucleotide sequence determination was performed by dideoxy-chain-termination procedure (Sanger et al., Proc.Natl.Acad.Sci., 74, 5463, 1977)

RESULTS

The pR plasmid protects bacteria and eukaryotic cells against UV and 4-nitroquinoline-N-1-oxide (4NQO) damages through the function of two regions, uvp1 and uvp2 (mucAB genes)(Marcucci et al., Mol.Cell.Biol., 6, 586, 1986). In a previous report (Elli et al., Mutation Res., 191, 177, 1987) we described the effect of pR plasmid on mammalian cells treated with methyl-N'-nitro-N-nitrosoguanidine (MNNG), bleomycin (BLM), cis-diamminedichloroplatinum (cis DDP) and mitomycin C (MMC), drugs that interact with DNA generating a broad spectrum of lesions, and we demonstrated that pR reduces the survival of transformed LTA cells after treatment with BLM and MNNG and, to a lesser degree, with cisDDP. Despite the very different way in which these drugs interact with DNA, there are two possible hypotheses to explain the sensitizing effect of pR on mammalian cells: 1) all the major lesions induced might be repaired by a postreplicative pathway that involves recombinative processes If recombination repair is important for the repair of cisDDP, BLM and MNNG damage and if the pR plasmid interacts with recombination events (Oliver, J. Gen.Microbiol., 101, 93, 1977, Brouwer et al., J.Bacteriol., 156, 1275, 1983, and Walker, Microbiol.Rev., 48, 60, 1974, reported discording data on the interaction of R46 and pKM101 plasmids with recombination repair in bacteria), these two phenomena might explain the pR sensitizing effect on mammalian cells treated with these drugs; 2) more likely, pR could interfere with mammalian short patch repair; in fact, damage caused both by alkylating agents and by ionizing radaiations is thought to be repaired via a short patch repair (different from the long patch repair of UV damage, Friedberg in 'DNA Repair' pp 323-374, 1985, Freeman Ed.). We have also demonstrated that the reduced survival consequent to the use of these drugs is really due to the function of the plasmid, because cells harbouring the pR plasmid mutated in the uvp2 region (LA-690 line) lack the sensitizing effect (Elli et al., 1987). In order to discover if the pR plasmid encodes proteins that exert the inhibitory effect interacting with the repair enzymatic system for recombination or for short patch repair, we investigated the function of the uvp1 region both in bacteria and in mammalian cells, using two different approaches.

Analysis of the expression of mutated pR plasmids in bacterial and in mammalian cells. The results obtained by analysing the survival curves of E. coli C600 transformed by pR w.t. and by pLM54 (a pR deleted in the uvp2 region) show that only the former protects against 4NQO and BLM damage, but it has no effect on cisDDP survival. The BLM resistance conferred by pR plasmid

is more apparent both when the drug concentration is increased and when time exposure is lengthened. BLM seems to be the most informative of the drugs, considering the oppositeeffect it has on bacterial cells and on mammalian cells. Therefore we investigated the effect of BLM on mouse cell lines transformed by different pR mutants obtained by the Tn5 insertion in the uvp1 region (LA-760 line) or in the uvp2 region (LA-690 line) or between these two regions (LA-542 line). The Tn transposon contains three antibiotic resistance gene(bleo, neo and str respectively for bleomycin, neomycin and streptomycin) controlled by the same promoter. The BLM and G418 (the eucaryotic equivalent of neo-kanamycin)resistance rates of the tested cell lines are the following : -the LA-D line, in which Tn5 transposon is not present is more sensitive to the damage induced by BLM and G418 than either untransformed line or lines transformed by pR::Tn5; -in the LA-760 line the neo and bleo genes expression eliminates the pR sensitizing effect; - in the LA-690 line the neo and bleo genes expression enhances the resistance to these drugs respect to LTA line; -in the LA-542 line the bleo gene expression partially eliminates the pR sensitizing effect.

Molecular characterization of the uvp1 region. We constructed a new plasmid pL11.5 (uvp1-, mucAB+) and analysed its expression in UV repair and UV induced mutagenesis. The UV survival curve of E.Coli cells harbouring pL11.5 plasmid is superimposable on that of untransformed cells, while its ability to increase the mutation rate following UV irradiation is not different from that of the pR plasmid. We demonstrated also that the uvp1 region encodes a protein (MW 20000) by comparing the electrophoretic pattern of the protein coded by the pLM54(uvp1+) plasmid with those coded by the pRES (uvp1-) plasmid. This datum confirms a previous identification of the same protein obtained with a pR mutant by Tn5 insertion in the uvp1 region (Gigliani et al.Nucleic Acid Res.623-631, 1981). The nucleotide sequence of 820 bp of uvp1 region contains an open reading frame of 594 nucleotides that corresponds to the protein of 20 kd. The uvp1 gene cooperates with the muc genes in repairing UV damagewithout being involved in UV-induced mutagenesis. A function of this gene could be to enhance the recombination activity of the cell as shown at least in two different genetic system tested: i) recombination of phage ams using a new genetic element, the pR plasmid (Battaglia et al.Mol.Gen.Genet., 209, 1987); ii)recombination rate in E.Coli KS391 which contains two copies of lac operon delcted in 2 non overlapping regions.

CONCLUSION

1) The uvp1 region contains a gene coding for a protein of 198 aminoacids which increases generalized recombination in bacteria and participates in UV repair by cooperating with the mucAB genes;

2) the ability of pR plasmid to sensitize mammalian cells to BLM is lost when the uvp2 region(mucAB genes) is mutated either by insertion or by deletion. The uvp2 region express the BLM sensitizing effect also when uvp1 region is mutated; however the uvp2 region expression is enhanced in presence of uvp1 region. These data confirm the cooperative function of the two regions in mammalian cells also.

PLASMIDS AND CELLS USED IN THF REPORTED EXPERIMENTS

TRANSFORMING DNA	MOUSF CELLS *
None	LTA
pTK1+pR wild type	LA-D
pTK1+pR 76S (uvp1-uvp2+)	LA-760
pTK1+pR 69S (uvp1+uvp2-)	LA-690
pTK1+pR 54R (uvp1+uvp2+)	LA-542
pSV2neo	LG1

TRANFORMING DNA	E.COLI STRAINS
None	C600
pR w.t.	C600 plus pR
pLM54 (uvp1+uvp2-del)	C600 plus pLM54
pRFS (uvp1- mucAB-)	C600 plus pRES
pL11.5 (uvp1-mucAB+)	C600 plus pL11.54

- pR (Ap+UV+) plasmid is an HindIII fragment 23kb pairs of TP120 and it confers UV resistance both in procaryotic and eukaryotic cells (Gigliani et al., 1981)
- pR76S -pR69S -pR54R are mutants of pR plasmid obtained by Tn5 insertions
- pLM54 is a mutant of pR obtained by deletion of uvp2 region(Marcucci et al., 1986)
- pRFS plasmid is a mutant of pLM54 obtained by deletion of uvp1 region
- pL11.5 plasmid is a derivative of pRES plasmid plus mucAb genes
- pTK1 (Ap+TK+) is derived from a 3.4 kb pairs fragment of HSV cloned into the BamHI site of pBR322 and it contains a selectable TK+ marker (kindly supplied by S.Bacchetti)
- pSV2neo is a plasmid derived by SV40 hybrid plasmid vectors; it contains a bacterial gene (neo) conferring resistance to neomicyn kanamycin antibiotics (kindly supplied by S.Bacchetti)

* Mouse cells are transfected by calcium phosphate method (Graham et al., Virology 456, 1973) and by electroporation method (Chu et al., Nucleic Acid Res. 15, 1311, 1987)

IV. Objectives for the next reporting period:

1)To evaluate the interaction between the function of the pR uvp regions and the expression of Tn5 transposon in BLM and genetycin responses of mouse cells transfected with pR w.t. and different Tn5 insertion mutants.
2)To evaluate the BLM response of human Ataxia Telangiectasia cells transfected with the pR plasmid, which increases the BLM sensitivity of 'repair proficient' mammalian cells. The AT lines used have been already characterized for the BLM sensitivity (Fiorilli et al.Hum.Genet.70, 274, 1985)and cotransfected with pR and pSV2neo plasmids by electroporation technique (Petrinelli et al.3°Congresso Nazionale FISME, 1988)
3)To characterize the pR plasmid integration sites in different cell lines.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Silvia Bacchetti- Mc Master University.Dept of Pathology, Hamilton, Ontario USA

M.Taylor- Indiana University.Dept.of Biology.Bloomington Indiana, USA

VI. Publications:

1) Elli R., Petrinelli P., Marcucci L., Proietti M., Bosi R., Antonelli A. Paradoxical effect of bleomycin on bacterial and eukaryotic cells transfected by a prokaryotic plasmid. Cytotechnology, suppl. August 1988, p19
2) Petrinelli P., Marcucci L., Antonelli A., Bosi R., Proietti M., Verni L. Giannantonio M.G., ELLI R. Trasfezione mediante 'electroporetion' di cellule di Atassia Telangiectasia con un plasmide procariotico che induce sensibilità alla bleomicina.3°Congresso Naz.Federazione Italiana per lo Studio delle Malattie Ereditarie (FISME) Ancona 1988
3)Battaglia P.A., Perri S., Sporeno E., Gigliani F. A 'controlling element' in Bacteria: the pR rep region. Proceeding of the Second Italy-Japan Joint Seminar on Biological Science P 35-36, Mondello (Palermo) 1988
4) Gigliani et al. The uvp1 gene of plasmid pR cooperates with mucAB genes in DNA repair process. In preparation

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-157-UK

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Head(s) of research team(s) [name(s) and address(es)]:

Prof. H.J. Evans
Clinical & Pop. Cytogenetics Unit
Western General Hospital
Crewe Road
GB - Edinburgh EH4 2XU

Telephone number: 031-332 2471

Title of the research contract:

Spontaneous and radiation-induced chromosome mutation and deletions of specific chromosome regions of the human karyotype which contain genes of known clinical importance.

List of projects:

1. An investigation of deletions of the short arm of chromosome 11 in man and the association of the deletions with the probability of developing Wilm's tumour.

Title of the project no.: Cytogenetic and molecular genetic studies of spontaneous and radiation-induced deletions of human chromosome 11, particularly with regard to their association with the development of Wilms' tumour and of other kidney and eye abnormalities

Head(s) of project: Professor H J Evans

Scientific staff: Dr N Hastie, Dr Veronica van Heyningen, Dr D Porteous, Dr Wendy Bickmore, Dr A Sharkey, Mrs Judy Fantes, Mr E Thomson

I. Objectives of the project:

To examine the distribution and consequences of spontaneous and X- and γ-ray-induced deletions in the short arm of human chromosome 11 and to identify and isolate genes that have undergone spontaneous or radiation-induced mutation.

II. Objectives for the reporting period:

These have been two-fold:
(a) To continue to map the 11p13-14 regions of the human genome and to locate HTF islands which are associated with the 5' ends of genes in these regions.
(b) By using pulse field gel electrophoresis techniques to examine the effects of X- and γ-radiation in inducing DNA fragmentation in the 11p13-14 chromosome region.

III. Progress achieved:

1. Methodology

(a) Cytogenetics using banded prophase preparations to define the chromosome 11p regions deleted in Wilms' tumour and aniridia patients.

(b) Segregation of mutated 11p chromosome arms from the normal chromosome 11 in human mouse hybrid cells and isolation of new DNA probes for the 11p13p14 region from these segregants and from murine cells subjected to chromosome mediated transfection with human chromosome 11.

(c) Pulse Field Gel Electrophoresis to isolate large DNA fragments to construct long range maps of 11p13 and 14 using the isolated DNA probes and previously isolated known gene markers.

(d) Size, and sequence content, determinations of DNA fragments induced by X and Co^{60} gamma irradiation of human lymphocytes. DNA fragments of chromosome 11, and other regions of the human genome, are identified with the isolated probes and sized using PFGE.

2/3 Results and discussion

The range of DNA probes that we have produced for the 11p13/14 region, coupled with pulse field gel electrophoresis, has enabled us to construct a long range map for this region. We have shown that the approximately 2 Mega bases of DNA encompassing that part of the chromosome between the gene for FSHβ and a sequence detected by our probe p4F11 contains 7 HTF islands (regions of hypomethylation that appear to mark the 5' end of genes). Moreover, we have found that the HTF islands are concentrated in the G-band negative 11p13 region, the G-band positive 11p14 band containing few such islands. The break points of the translocation associated with Potter facies and genitourinary dysplasia, and which map within the smallest region of overlap as defined by our Wilms' tumour-aniridia-genitourinary-mental retardation (WAGR) deletions, is located within one of these 7 putative genes. Progress has been made in 'walking' along the region of interest towards the translocation break point and our data indicate that we are now within ~250 kb of the Wilms' gene. We have shown that the translocation break point cluster at 11p13 which is associated with human T cell acute lymphocytic leukaemia is located near to, but outwith, the Wilms' tumour locus.

A part of our approach is to examine the effects of ionising radiations in inducing mutations in a genetically well-defined region of the human genome, and indeed to use such radiations as tools to dissect the structure of the genome. To this end lymphocytes have been irradiated with a range of X- or γ-ray doses and the extracted DNA analysed for fragment size using pulse field gel electrophoresis and appropriate DNA probes. We have found that the size distribution of the radiation-induced DNA fragments is not random. Further studies are in progress to identify the underlying cause of this non-uniformity and to relate the sites of radiation-induced breakage to specific chromosome domains and coding sequences.

IV. Objectives for the next reporting period:

1. To characterise, and where appropriate to clone in YAC vectors, human DNA fragments produced as a consequence of radiation exposure.

2. Hopefully, to finally isolate the Wilms' tumour locus and to assay, using mouse cell transgenomes, for the tumour suppressing activity of the sequences in this locus.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr Claudine Junien, Paris.
Dr G Fekete, Semmelweis Medical School, Budapest, Hungary.
Dr Alan Balmain, Beatson Institute, Glasgow.
Dr K O J Simola, University of Helsinki, Finland.
Dr Jane Fennell, Royal Manchester Children's Hospital, Manchester.
Dr R Carachi, Royal Hospital for Sick Children, Yorkhill, Glasgow.
Dr A Pearson, Newcastle.
Dr E Stanbridge, Department of Microbiology and Molecular Medicine, University of California, Irvine, USA.
Dr T Rabbitts, MRC Laboratory of Molecular Biology, Cambridge.

VI. Publications:

Bickmore W, Christie S, van Heyningen V, Hastie N D and Porteous D J. *Nucleic Acids Res* **16,** 51-60, 1988.

Bickmore W A and Hastie N D *Ophthal Paediat Genet* (in press).

Hastie N D, Porteous D J, Bickmore W, Maule J and van Heyningen V **IN:** *Genetics of Immunological Diseases* (ed B Mock and M Potter), pp 41-46, Springer-Verlag, Berlin, 1988.

Seawright A, Fletcher J M, Fantes J A, Morrison H, Porteous D J, Li S S-L, Hastie N D and van Heyningen V. *Somat Cell Molec Genet* **14,** 21-30, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D - 8042 Neuherberg

Contract no.: BI6-E-159-D

Head(s) of research team(s) [name(s) and address(es)]:

Dr. D. Frankenberg
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GSF
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D - 6000 Frankfurt/Main

Telephone number: 069-6303310

Title of the research contract:

RBE-values of monoenergetic electrons for DNA double-strand breaks, chromosome aberrations and lethality and point mutations.

List of projects:

1. Monoenergetic low energy electron irradiation of yeast and mammalian cells to study the induction of cell killing, aberrations, mutations and DNA double strand breaks as a function of electron energy.

Title of the project no.: 1

Monoenergetic low energy electron irradiation of yeast and mammalian cells to study the induction of cell killing, gene conversion, mutations and DNA double strand breaks as a function of electron energy

Head(s) of project:

Priv.Doz.Dr.D.Frankenberg

Scientific staff:

Priv.Doz.Dr.D.Frankenberg

Dr.H.Kühn

Dr.M.Frankenberg-Schwager

I. Objectives of the project:

RBE-values will be determined for the induction of DNA double strand breaks (DSB), the induction of gene conversion and reversion, and lethality as a function of electron energy using characteristic ultrasoft X-rays. With the help of synchronized cells of the mutant rad54-3 (temperature conditional for DSB repair) the fate of DSB in cells proceeding through the cell cycle will be investigated.

II. Objectives for the reporting period:

1. Dose rates of characteristic ultrasoft X-rays as a function of atomic number Z of target material

2. Inactivation and induction of DNA double strand breaks (DSB) by Al_K characteristic X-rays: A comparison between the facilities in Frankfurt and Harwell.

3.Gene conversion and mutation (reversion) in yeast after 25 MeV electron irradiation as a reference radiation for the investigations with ultrasoft X-rays. Possible correlations between repair of DSB and gene conversion and reversion, respectively, are evaluated.

III. Progress achieved:

1. The proton-induced characteristic X-rays penetrate different materials before entering the biological objects: The window between vacuum and driftspace, the driftspace itself and a thin foil as a support of the cells to be exposed. Considering the attenuation by these materials, the dose rate in air as a function of Z with different gases in the driftspace was calculated and is shown in figure 1. The experimental data obtained with C_K, Al_K, Ti_K and Cu_K are in satisfactory agreement with the calculated values. It can be concluded that characteristic X-rays of target materials with Z higher than 17 are too low in dose rate as to be used in biological experiments.

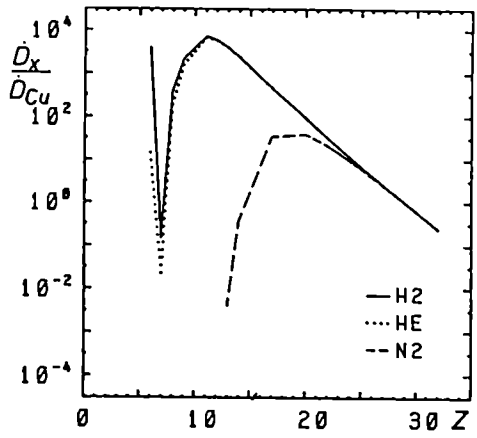

Figure 1 : Normalized dose rates in air of characteristic ultrasoft X-rays as a function of the atomic number Z of target material. The relative dose rate of Cu_K is normalized to 1. Parameters are the gases of the drift space.

2. Based on the dosimetry and spectrometry of Al_K characteristic X-rays an exponential survival curve was obtained for rad54-3 cells when DSB repair was inhibited by incubation of irradiated cells at 36°C. The D_o-value agrees within 5% with that determined in Harwell for these cells. The RBE-value relative to <5 MeV electrons for DSB induction amounts to 2.5.

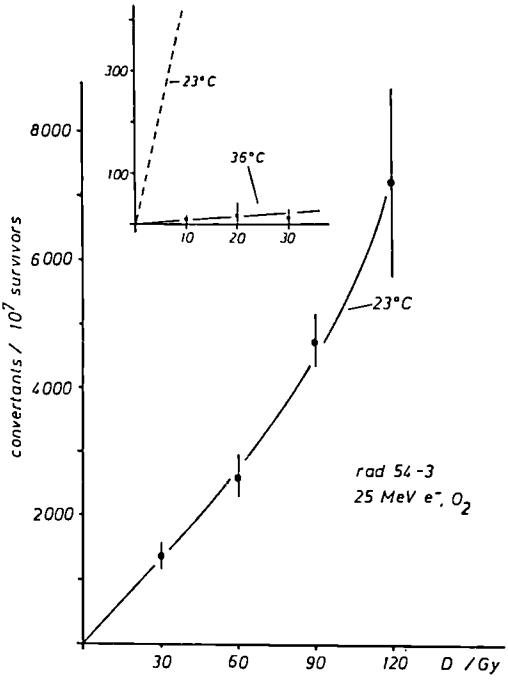

Figure 2 : Induction of gene conversion in rad54-3 cells incubated at 23°C or 36°C after irradiation with 25 MeV electrons under oxic conditions

3. For the evaluation of RBE-values of C_K and Al_K X-rays for gene conversion and reversion, these endpoints were investigated with 25 MeV electrons as a reference radiation using rad 54-3 cells. Since this strain is deficient in DSB repair at 36°C and proficient at 23°C, a possible correlation between gene conversion or reversion and DSB repair can be investigated. Figure 2 shows that the yield of convertants is linear with dose up to 60 Gy and supralinear for doses higher than 60 Gy. The yield of convertants in cells incubated after irradiation at 36°C is by a factor of about 70 smaller relative to cells incubated at 23°C showing that gene conversion is correlated with repair of DSB. In contrast, the yield of revertants is not significantly different between rad54-3 cells incubated at 36°C or 23°C after 25 MeV electron exposure.

IV. Objectives for the next reporting period:

Dosimetry and spectrometry of F_K (0.676 keV) characteristic X-rays will be performed. Induction of DSB by these ultra-soft X-rays will be determined.
RBE-values of C_K and Al_K characteristic X-rays for gene conversion and reversion will be evaluated relative to 25 MeV electrons.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Internal reports:

H.Kühn und D.Frankenberg: Ultraweiche charakteristische Röntgenstrahlen, Jahres bericht 1987, Gesellschaft für Strahlen- und Umweltforschung, München, 138-144 (1988)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-E-160-B

Univ. Catholique Louvain-la-Neuve
Place de l'Université 1
B - 1348 Louvain-la-Neuve

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 010-47 36 14

Title of the research contract:

The role of recombination in yeast mitochondrial DNA repair.
Influence of ionizing radiation.

List of projects:

1. The role of recombination in yeast mitochondrial DNA repair.
Influence of ionizing radiation.

Title of the project no.:

The role of recombination in yeast mitochondrial DNA repair. Influence of ionizing radiation.

Head(s) of project:

A. Goffeau

Scientific staff:

F. Foury
A. Lahaye
E. Van Dyck

I. Objectives of the project:

Our research objective aims to a better understanding, at the molecular level, of the repair of mitochondrial DNA (mtDNA) in the yeast Saccharomyces cerevisiae after irradiation, mainly by gamma rays. Our approach consists in isolating mutants deficient in repair, to clone the corresponding genes by genetic complementation of the mutated alleles and to sequence them, and finally to try to determine the biochemical function of the gene product by various strategies.

II. Objectives for the reporting period:

The PIF1 product involved in repair and recombination of mitochondrial DNA has been overexepressed in yeast and in bacteria in order to try to determine its enzyme function, more specifically, whether it has nuclease and/or DNA unwinding (DNA helicase) functions.

III. Progress achieved:

We have established that the nuclear gene PIF1 in Saccharomyces cerevisiae encodes a 97.5 kda protein imported into the mitochondria, whose structure is closely related to that of a broad family of DNA helicases which share six conserved motifs along the amino acid sequence. The PIF1 protein is strictly required: 1) for the stimulation of mtDNA recombination by a recombinogenic signal; 2) for the repair of mitochondrial DNA after treatment with various mutagens, including UV light and gamma rays; 3) for the maintenance of mtDNA after treatment of the cells at elevated temperature (production of more than 99.9% cells devoid of mitochondrial DNA at 36.5°C). These results suggest that repair of mtDNA in S. cerevisiae operates through recombination, which should be a major repair pathway. Therefore, the finding that PIF1 protein exhibits sequence similarities with the RecB, RecD and RecC polypeptides of ExoV in E. coli of special interest. Thus multifunctional enzyme has a DNA unwinding activity requiring the RecB polypeptide, and a nuclease activity involving RecC and RecD polypeptides. It is a major enzyme in E. coli recombination, which specifically recognizes the Chi sequence, and it plays an important role in post-replication repair. RecB and RecD polypeptides belong to the helicase family, while RecC has an unrelated structure. The related structure between PIF1 protein and the RecBCD complex suggests that they might have related functions. Our goals was therefore to determine the enzyme activities of PIF1 protein.

As the PIF1 protein is in extremely low concentration in the cell, it was necessary to overexpress it in yeast and to devise an assay to detect it in yeast extracts. First, the gene PIF1 was fused to the lacz gene and placed under the control of the thermoinducible promoter Pr of phage lambda. An antiserum against the overexpressed fusion protein was obtained in a goat and used for immunological detection of the PIF1 protein in yeast. The PIF1 protein was overexpressed in yeast, by placing the PIF1 gene under the control of the strong inducible GAL1 promoter, on a plasmid containing a high

number of copies. The plasmid was introduced into a nuc1 null strain (devoid of the major mitochondrial DNA nuclease activity), in order to facilitate the DNA helicase assays. It was verified that the overexpressed protein is imported into the mitochondria, by introducing the plasmid in a conditional mutant (mas1) deficient in mitochondrial import: at 37°C, a precursor accumulated, whose molecular weight was higher than the overexepressed protein at the permissive temperature. Moreover, the overexpressed protein is functional as demonstrated by complementation of a pif1 null mutant in vivo. Finally, it was found that the overexpressed protein is membrane bound and solubilized by zwittergents in the presence of KCl. We are now ready to measure the putative enzyme activities.

IV. Objectives for the next reporting period:

1) We will start the purification of the overexperessed PIF1 protein, using classical ion exchange and affinity chromatographies and we will develop the DNA helicase assay.

2) We will start the cloning of additional pifx mutants which have the property to be functionally complemented by the PIF1 gene present in multiple copy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

- A. Lahaye and F. Foury (1988) Overexpression of PIF1 gene involved in recombination and repair of mitochondrial DNA of S. cerevisiae (14th International Conference of Yeast Genetics and Molecular Biology, Helsinki), in Yeast, Vol. 4, p 227.

- E. Van Dyck and F. Foury (1988) A nuclear gene involved in mitochondrial DNA metabolism (14th International Conference of Yeast Genetics and Molecular Biology, Helsinki), in Yeast, Vol. 4, p 237.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

University College
IRL - Galway

Contract no.: BI6-E-162-IRL

Head(s) of research team(s) [name(s) and address(es)]:

Prof. J.A. Houghton
Department of Microbiology
University College
IRL - Galway

Telephone number: 091-24411

Title of the research contract:

A study of the effects of radiation on the chromosomes of human gametes.

List of projects:

1. A study of the effects of radiation on the chromosomes of human gametes.

Title of the project no.: B16-E-162-IRL

A Study of the Effects of Radiation on the Chromosomes of Human Gametes.

Head(s) of project: Professor James A Houghton
Department of Microbiology
University College
Galway
Ireland

Scientific staff: Dr P Tomkins, Dr S Houghton, Ms C Gillespie.

I. Objectives of the project:

The primary objective of this work is to obtain genetic information on the deleterious effects of radiation on the paternal germ line by direct analysis of human sperm chromosomes. To achieve this, the following studies have been undertaken: (i) the development of new sperm/egg fusion technology and culture techniques to enable the sperm chromosome assay to attain the level of efficiency necessary for application to radiation protection studies; (ii) application of novel banding methods and computer enhancement to the study of sperm chromosomes; (iii) study of sperm damage at the membrane and genome level following irradiation; (iv) the study of sperm cell systems involved in radiation protection.

II. Objectives for the reporting period:

(1) Develop electropermeabilization for acrosome reaction (AR) induction prior to sperm/egg fusion for sperm chromosome analysis and increase sensitivity to external Ca^{2+} using ionic pulse media and intracellular secondary messengers. (2) Assess the chromosome protective value of extracellular superoxide dismutase, catalase and taurine to fertilized eggs. (3) Study the consequences of the inhibition of sperm intracellular SOD on sperm function, lipid peroxidation and in vitro radiation tolerance. (4) Examine the effects of phenothiazine drugs on AR induction and chromosome formation. (5) Study more radiotherapy patients to further analyse the effects of radiation exposure on sperm chromosomes.

III. Progress achieved:

(1) Successful sperm chromosome analysis (SCA) is dependent on efficient fusion of donor sperm and zona-free hamster ova. However, sperm from males who have received radiotherapy up to 5 y previously are severely disturbed, reducing the possibility of data from the traditional SCA. Oligoasthenozoospermic products of such treatments exhibit poor survival in culture and a significantly reduced probability of undergoing a normal acrosome reaction (AR). Men who have received combined radio- and chemotherapy are more likely to demonstrate a sustained or permanent period of azoospermia. The success rate for producing analyzable sperm metaphases ranges from 2-60% for normal samples and 0-3% for radiotherapy samples. The application of electropermeabilization to sperm has enabled rapid assisted calcium ion uptake to induce a uniform fusiogenic status in sperm with intact acrosomes and equatorial segments. Cells are exposed to a high voltage pulse in the presence of a Ca^{2+} containing medium and then washed before mixing with eggs. This technology has led to a significant increase in the success of SCA and has enabled the study of fundamental aspects of sperm physiology and biochemistry. Furthermore, it has become apparent that modification of the pulse medium will benefit analysis of subfertile sperm from radiotherapy patients. Electropermeabilization of sperm was initially demonstrated using covalent pulse media. However, such media are incompatible with fertilization and the sperm must be transferred to culture media prior to gamete interaction, with consequential cell damage. The advantage of an ionic medium is that it permits simple post-pulse dilution without washing. A study was, therefore, carried out on several ionic media formulations, all of which contained a polymer and Ca^{2+}. Within this group, the most successful was HEPES buffered KCl-inositol. For normospermic samples it gave within 80% of the fusion response of pulsing in sucrose and for oligooospermic samples it proved superior.
For exocytotic systems, it has been proposed that phospholipid breakdown may be the initial event that follows receptor activation. This breakdown liberates DAG and inositol phosphates, IP2 and IP3, which may function as intracellular messengers and mobilize the release of intracellular calcium stores and mediate Ca^{2+} uptake. We have demonstrated that sperm pulsed in a partially ionic medium in the presence of IP3 exhibit significantly enhanced sensitivity to extracellular Ca^{2+}. In the presence of $\leq$ 0.75 mM Ca^{2+}, the addition of IP3 induces an 85% increase in the penetration response and in the presence of 10 mM Ca^{2+} it yields a 26% increase in the level of polyspermy. Whether these results are due to increased utilization of pulsed Ca^{2+}, increased uptake or release of intracellular Ca^{2+} is not clear, but this is the first report of an IP3 induced effect on sperm exocytosis.

(2) Mitomycin C is known to induce preferential breaks in constitutive heterochromatin and this is thought to be finally mediated by superoxide anions. It thus shows some similarity in mechanism to the genotoxic action of radiation. We have previously shown that in vitro irradiation of sperm in the presence of superoxide dismutase (SOD) and catalase can attenuate most of the deleterious effects on sperm associated with irradiation alone. We have demonstrated that incubation of heterospecifically penetrated eggs from S to M phase in simple deinfed culture medium containing mitomycin C leads to a high index of clastogenic damage in the resultant sperm metaphases, with up to 20% of human-hamster cells

exhibiting chromatid breaks. Initial analysis suggests that inclusion of SOD and catalase in the culture inhibits the activity of mitomycin C and returns break counts to 1-2%: near the zero level of typical controls.

(3) A plot of seminal plasma SOD activity against post-irradiation therapy time has provided tentative evidence that this enzyme might be induced in testicular compartments by clinical levels of ionizing radiation. There was no correlation between SOD and sperm motility or performance in the penetration assay, but it was positively correlated with seminal plasma zinc. UV irradiation of sperm in vitro in polymer-free defined medium gives a characteristic survival curve in terms of motility, with an initial 20% mobility declining to zero about 3 h after irradiation. Inclusion of SOD in the medium during and after treatment led to an approximate 30% increase in motile survival, while the further presence of catalase led to an average increase of 65% over 5 h. When sperm SOD is selectively inhibited with DDC there is no survival following UV and addition of radical scavengers does not improve the performance. Exposure to DDC in protein-free, polymer-containing medium results in the elimination of motility within 2 h. Decreased motility is maintained in the presence of protein but surface adhesion occurs within 3 h. The decline in motility was not associated with a significant rise in lipid peroxidation or loss in viability.

(4) Many of the calcium-induced responses in sperm are mediated by calcium binding proteins. It has been suggested that calmodulin might be the intracellular receptor for Ca^{2+} in the AR. To study this, sperm were treated with antipsychotic phenothiazine calmodulin-binding drugs. Their effect could be overridden by the pulse procedure, but following pre-incubation methodology, there was a small but significant increase in the penetration score following incubation with >= 10 uM of the drug while inhibition occurred at <= 1 uM. The differential response of two of the drugs (TFP and PMZ) is tentative evidence for the involvement of calmodulin or synexin-like proteins.

SUMMARY

(1) The electropermeabilized induction of the sperm AR has been further enhanced by the development of a new partially ionic pulse medium that increases calcium sensitivity without compromising cell motility. This development will further benefit the analysis of the effects of radiation on sperm chromosomes.

(2) The deleterious effects of mitomycin C on sperm chromosomes can be effectively attenuated by co-treatment with SOD and catalase.

(3) Sperm and accessory gland levels of SOD may be induced following irradiation. Seminal plasma SOD does not correlate with any seminal parameter except zinc. Addition of SOD and catalase can preserve sperm motility following UV provided the intracellular enzyme is still functional. Inhibition of intracellular SOD leads to a rapid decline in motility in the absence of protein and a decline in the quality of motility in the presence of protein which is not associated with an increase in lipid peroxidation.

(4) Phenothiazine drugs exhibit a concentration threshold for stimulation and inhibition of the sperm AR which in the absence of other membrane effects would implicate calcium binding proteins.

IV. Objectives for the next reporting period:

An effective technique has been developed for sperm chromosome analysis from radiation-exposed males which relies on the fusion of electropermeabilized sperm with hamster ova. The laboratory now has access to more sophisticated pulse equipment and improved sperm treatment methods will be introduced. This technology will be used in the study of sperm chromosome abnormality in radiotherapy patients. Patient numbers have been depleted over the last two years by further surgery, azoospermia and poor cooperation. As well as recruiting further patients, it is intended to analyse past patient records and design a retrospective study based on suitable men who completed radiotherapy treatment five and ten years previously.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr M Hurley
Radiotherapy Unit
Regional Hospital
Wilton
CORK

Dr M Moriarty
St Luke's Hospital
DUBLIN

Dr D Cannon
Mater Misericordiae
Hospital
DUBLIN

VI. Publications:

Tomkins, P.T., Carroll, C.V. and Houghton, J.A. (1988) Assessment of heterospecific zona-free ovum penetration under fully defined conditions. Human Reproduction 3, 367-376.

Tomkins, P.T. and Houghton, J.A. (1988) The rapid induction of acrosome reaction of human spermatozoa by electropermeabilization. Fertility and Sterility 50, 329-336.

Tomkins, P.T. and Houghton, J.A. (1988) Seminal plasma zinc and superoxide dismutase in irradiated and suspected subfertile males: relationship with seminal parameters and *in vitro* radioprotection. (In press).

Tomkins, P.T. and Houghton, J.A. (1988) Pronuclear chromosomes obtained following electrically assisted induction of the acrosome reaction of sperm. Presented at the Genetics Society of Ireland Meeting, Galway, April 1988.

Houghton, J.A. (1988) The study of human sperm chromosomes and the application of image-analysis techniques. Presented at the 10th European Workshop on Automated Cytogenetics, Llangollen, September 1988.

Tomkins, P.T., Carroll, C.V. and Houghton, J.A. (1988) The application of multiple banding techniques, alone and in conjunction with image analysis, to the study of human sperm chromosomes. Heredity 60, 313-314.

Tomkins, P.T. and Houghton, J.A. (1988) The value of sperm cell systems in toxicity assessment. Presented to the Irish Society of Toxicology, Athlone Meeting in March 1988.

Tomkins, P.T. and Houghton, J.A. (1989) Seminal plasma superoxide dismutase: relationship with seminal parameters and in vivo and in vitro radioprotection. Consequences of inhibition of the intracellular enzyme. Int. J. Androl. (Submitted).

Tomkins, P.T. and Houghton, J.A. (1989) The effect of pulse medium composition on the electropermeabilized induction of the sperm acrosome reaction. Gamete Res. (Submitted).

Tomkins, P.T. and Houghton, J.A. (1989) The effect of sperm pre-treatment with phenothiazines on the fusion response in the defined sperm penetration assay. Andrologia. (In preparation).

Tomkins, P.T., Carroll, C.V., Gillespie, K. and Houghton, J.A. (1989) The visualization of human sperm chromosomes and evidence for paternal DNA repair in a heterospecific environment under chemically defined conditions. (In preparation).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für
Schwerionenforschung
Planckstrasse 1
D - 6100 Darmstadt

Contract no.: BI6-E-197-D

Head(s) of research team(s) [name(s) and address(es)]:

Dr. G. Kraft
Angewandte Forschung/Biologie
GSI
Planckstrasse 1
D - 6100 Darmstadt

Telephone number: 06151-359.607

Title of the research contract:

Genetic changes in mammalian cells following heavy ion irradiation.

List of projects:

1. Genetic changes in mammalian cells following heavy ion irradiation.

Title of the project no.:

Genetic changes in mammalian cells following heavy ion irradiation

Head(s) of project:

G. Kraft

Scientific staff:

W. Kraft-Weyrather
S. Ritter
M. Scholz

I. Objectives of the project:

Heavy ion beams from the heavy ion accelerators UNILAC (Darmstadt) and GANIL (Caen), in the energy range between 0.3 MeV/u and 100 MeV/u, will be used to study the mechanisms of the action of heavy charged particles on mammalian cells.

The induction of chromosome aberrations, changes in cell cycle and radiosensitivity of V79 Chinese hamster cells will be studied as a function of radiation quality (atomic number, energy and LET), the fractionation of the exposure, the time after exposure and the cell cycle stage during exposure.

II. Objectives for the reporting period:

1. To measure the induction of chromosome aberrations in synchronized V79 Chinese hamster cells after heavy ion irradiation.

2. To investigate changes in cell cycle progression.

3. To measure the cell cycle dependence of radiosensitivity.

4. To start chromosome analysis using cytofluorometric methods.

III Progress achieved

V79 Chinese hamster cells have been irradiated with 10.5 MeV/u- Ar-ions (LET = 1100 keV/μm), 14 MeV/u-Ni-ions(2000 keV/μm), 4 MeV/u-Ti-ions (2500 keV/μm), 10 MeV/u Pb-ions (LET = 13500 keV/μm), 5 MeV/u U-ions (15000 keV/μm) and 14 MeV/u U-ions(14000 keV/μm) at the UNILAC, Darmstadt, and with 45 MeV/u Ca-ions (400 keV/μm) and 79 MeV/u Ar-ions (330 keV/μm) at the GANIL, Caen. In these experiments, the induction of chromosome aberrations, cell cycle perturbations and cell survival have been measured. In addition to asynchronously growing cell populations, synchronous V79 cells were prepared by centrifugal elutriation according to a standard protocol (Radiation protection, Progress Report, 1987, 1143-1148). For the experiments at the GANIL, synchronous cells were frozen and thawed without major loss of synchrony.

1. Induction of chromosome aberrations

The induction of chromosome aberrations by different ions is compared for similar survival levels. In order to interprete the time course of the expression of chromosome aberrations, studies of cell cycle effects are necessary. Since irradiation leads to drastic delays in the cell cycle, fewer cells proceed to mitosis where chromosomes can be observed.
The induction of chromosome aberrations has been measured for asynchronous and synchronous cells. Up to now, however, only the metaphases of asynchronous cells have been analyzed in detail, since the complexity of the analysis makes it more time consuming and difficult than previously estimated.
In order to determine absolute cross sections for the induction of chromosomal aberrations it is necessary to include the time course of the mitotic index after irradiation. Usually, a maximum of chromosome aberrations per 100 metaphases can be scored about 8 hours after irradiation. In this time interval the mitotic index is still significantly reduced, however, and a only few cells proceed through the cell cycle and reach mitosis (fig. 1). This is especially true for exposure to low energetic particles where much longer delays have been observed in all stages of the cell cycle
In order to compare the relative yields of aberrations under various conditions the number of aberrant cells per 100 metaphases is multiplied with the mitotic index and plotted as a function of time (fig 2) In all three cases shown here the yield of aberrant cells is increasing with time and reaches a plateau value within the first 24 hours. However, the high energetic Ar-ions having lower LET values are close to the X-ray curve, while the low energetic Ar-ions corresponding to high LET values seem to produce fewer damaged cells. This is due to the fact that high LET radiation introduces more complex lesions which arrest the cell in interphase (see section III 3) so that cells do not reach mitosis. Therefore, a fraction of the primary damage introduced by high LET radiation will not be scored at mitosis The difference in the damage produced by high and low LET radiation is also evident from the fact that in both cases approximately the same degree of cell survival has been observed, however less chromosome damage has been found for the high LET radiation.

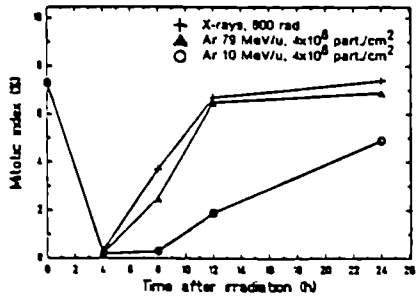

Fig 1

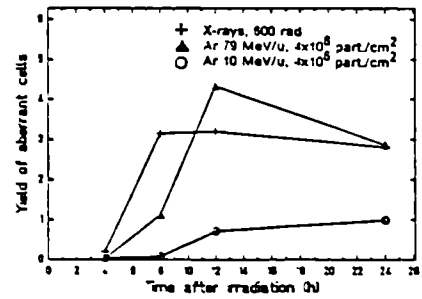

Fig. 2

2. Cell cycle progression

Perturbations in the cell cycle are analyzed by flow cytometric measurements of the DNA content of cells up at 1.5 h intervals up to 50 hours after irradiation. For all experiments synchronized cells were used, which have the advantage that absolute delay effects can be measured for cells in different cycle stages. In contrast, using asynchronously growing cells, it is only possible to determine relative changes in cycle progression for cells in different cycle stages.

In fig. 3, the result of an exposure to 10 MeV/u Pb-ions (LET = 13500 keV/μm) is compared with an X-ray experiment using cell samples with a similar survival level (ca. 25%). Synchronous G_1-cells were irradiated with $2x10^6$ particles/cm^2 and 600 rad X-rays. Synchronized, unirradiated control cells continue to progress through the cell cycle, giving rise to the oscillating curves seen in fig. 3a. The irradiation with Pb-ions drastically reduces the proliferation capacity of cells (fig. 3b): Firstly, the exit time out of the G_1-phase is about 2-3 times longer than for control cells. Secondly, many cells are obviously arrested in S- and G_2M-phase and do not proceed to mitosis. However, a small but increasing fraction of cycling cells can be observed in the G_1- and S-compartment, which may be due to the statistics of particle exposure: at an average of 2 hits per cell nucleus, 15% of the cell nuclei will not be hit by a particle at all and these will proliferate via the normal cycle time.

These distinct delays are completely different from the effects observed after exposure to X-rays. If cells are X-irradiated in G_1-phase, the passage of cells out of G_1- and through the S-phase is only slightly delayed. The only significant delay is in the G_2M-phase (see fig. 3c). One possible explanation for the observed differences between heavy ion and X-ray irradiation could be, that high LET radiation causes more severe damages than sparsely ionizing radiation. This means that more cells have already died in the first cell cycle, during interphase, before the first mitosis is reached. However, these cells are still detectable, since it takes time before they eventually disintegrate.

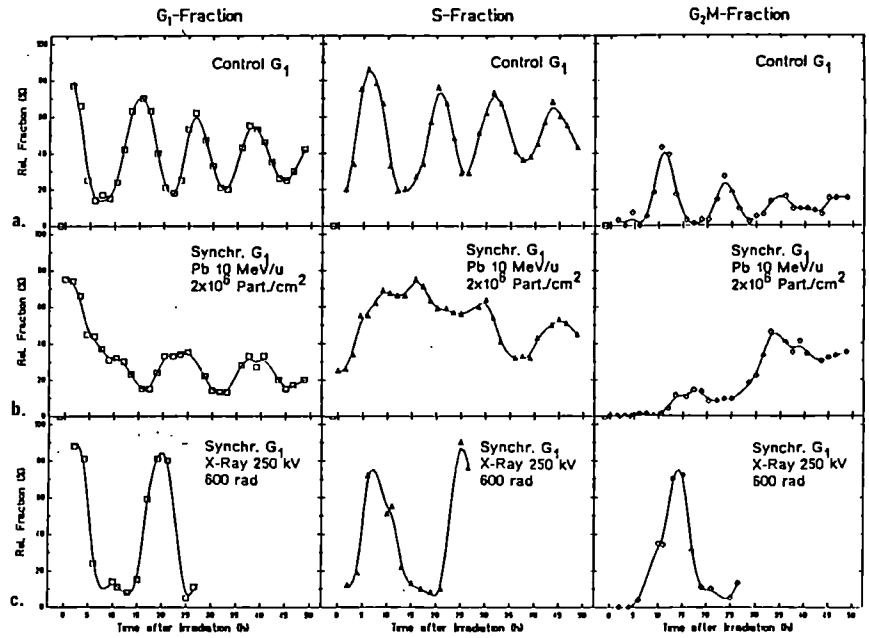

Fig. 3
a. Proliferation of unirradiated synchronous G_1-cells
b. Proliferation of synchronous G_1-c[illegible] exposure to 10 MeV/u Pb-ions
c. Proliferation of synchronous G_1-c[illegible] sure to 600 rad X-rays

3. Cell cycle dependent radiosensitivity

In several exeriments the cell cycle dependent radiosensitivity of V79-cells was measured. After synchronization, cells were plated in Petri dishes, allowed to attach, then irradiated, trypsinized, counted and plated for survival.
These experiments yielded unexpected results when compared to the well known cell cycle dependence of X-ray radiosensitivity. For heavy ions, radiosensitivity is lowest for G_1-cells. Radiosensitivity increases through the rest of the cycle, with maximum cell sensitivity in late S phase or at the S/G_2-boarder (fig. 4). In the late phases of the cell cycle (late G_2 and M) radiosensitivity again decreases. This pattern of radiosensitivity, which is largely opposite to the cell sensitivity to X-rays, has been observed in all experiments with synchronous cells up to now and was more significant for the heavier ions. The increasing sensitivity might be attributed to geometrical effects (i.e. increase of the geometrical cross section of cell nuclei during the cell cycle) and changes in DNA conformation when cells prepare for division.

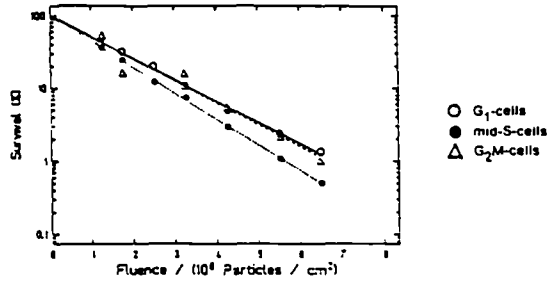

Fig. 4
Survival curves of synchronous G_1-, S- and G_2M-cells exposed to 10 MeV/u Pb-ions

4 Chromosome analysis using flow cytometry

The first experiments have been performed to isolate chromosomes from cells arrested in mitosis in order to obtain a suspension of chromosomes for flow cytometric measurements. The best resolution in chromosome distributions achieved up to now is shown in fig. 5. However, it has not yet been possible to set up a standard protocol which always yields reproducible results under the difficult conditions imposed by an accelerator experiment.

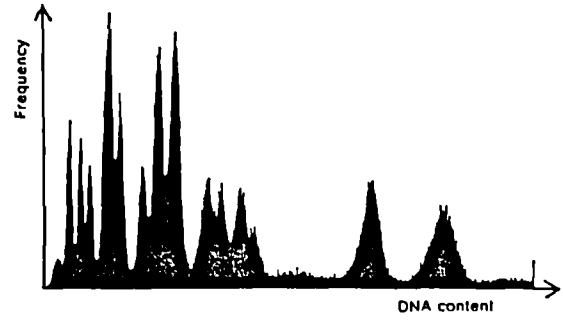

Fig 5
DNA content distributions of the chromosomes of V79-cells

IV. Objectives for the next reporting period:

Measurements of heavy ion induced chromosome aberrations, changes in cell cycle progression and differences in the radiosensitivity of cells in different phases will be continued using synchronous cells.
A standard protocol for chromosome analysis using cytofluorometric methods will be developed.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

E.A. Blakely, R. Roots, C.A. Tobias
LBL, Berkeley, USA

B. Dutrillaux, L. Sabatier,
CEA-IPSN, Fontenay-aux-Roses, France

VI. Publications:

Kraft, G.:
Effects of LET, fluence and particle energy on inactivation, chromosomal aberrations and DNA strand breaks.
In: P.D. Mc Cormack, E. Swenberg and H. Bücker (eds.), Terrestrial space radiation and its biological effects, Plenum Publishing Corporation, New York, pp 163-184, 1989.

Ritter, S. and G. Kraft:
Induction of chromosome aberrations in Chinese hamster cells after heavy ion irradiation.
In: P.D. McCormack, E. Swenberg and H. Bücker(eds), Terrestrial space radiation and its biological effects, Plenum Publishing Corporation, New York, pp 185-191, 1989.

Kraft, G.:
On the Interpretation of radiobiological experiments performed with heavy charged particles.
GSI Preprint 88-33,
To be published as Technical Report of IAEA, Vienna

Kraft, G., W. Kraft-Weyrather, S. Ritter, M. Scholz and J. Stanton:
Cellular and subcellular effect of heavy ions: A comparison of the induction of strand breaks and chromosomal aberrations with the incidence of inactivation and mutation.
GSI Preprint 88-52
To be published in Adv. in Space Res.

Scholz, M., W. Kraft-Weyrather, S. Ritter and G. Kraft:
Cell cycle delays induced by heavy ion irradiation of synchronous mammalian cells.
GSI Preprint 88-53
To be published in Adv. in Space Res.

Kraft, G.:
Radiobiological experiments with heavy ions. A comparison of the cross section of different biological endpoints.
GSI Preprint 88-58,
Proceedings of the workshop on " Mutagenic effects of heavy ions" , Dubna, 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre d'Etude de l'Energie Nucl.
CEN/SCK
Rue Charles Lemaire, 1
B - 1160 Bruxelles

Contract no.: BI6-E-146-B

Head(s) of research team(s) [name(s) and address(es)]:

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Department of Radiobiology
CEN/SCK
Boeretang 200
B - 2400 Mol

Telephone number: 014-31.18.01

Title of the research contract:

Radiation-induced structural chromosome aberrations in mammalian somatic cells.

List of projects:

1. Chromosome aberrations in peripheral blood lymphocytes of radiation therapy patients measured as biological indicators of genetic damage in man after partial body irradiation.
2. Accurate estimation of dose effect relationship for chromosome aberrations induced in human lymphocytes at low doses of X rays.

Title of the project no.: 1

Chromosome aberrations in peripheral blood lymphocytes of radiation therapy patients measured as biological indicators of genetic damage in man after partial body irradiation.

Head(s) of project:

Alain LEONARD

Scientific staff:

Ghislain DEKNUDT
Paul JACQUET

I. Objectives of the project:

Dose-effect relationships for the induction of chromosome aberrations have been well established for irradiation in vitro but are still relatively scarce after in vivo inhomogeneous exposure such as expected after accidental irradiation. For that purpose information can be obtained from patients undergoing therapy under well controlled technical conditions. An advantage of this procedure is that the patients provide their own control and that chromosomal aberration yields can be followed in the same person, after increasing dose levels.

II. Objectives for the reporting period:

Cytogenetic observations were made on patients receiving highly localized γ rays for glioblastoma.
In addition, some studies have been completed on the persistence of chromosome aberrations in accidentally irradiated subjects.

III Progress achieved

a. Chromosome aberrations in patients irradiated for glioma

1. Methodology

Among the tumors of neuroglial origin, astrocytomas exemplify a progression of malignancy from benign (low grade) toward a highly malignant type (astrocytomas grade IV, glioblastomas). The surgical tumor resection is very often incomplete and, at the present time, some improvement of the postoperative survival can only be obtained by radiation therapy. To be effective, a total dose of X-rays (linear accelerator) or γ-rays (Co-60 unit) as high as 5,500 cGy must be delivered to a large volume. The patients were treated with a Co-60 unit (Picker) according to the following protocol :

- a large homolateral field, to encompass the whole cerebral hemisphere corresponding to the tumor ;
- two opposed lateral fields limited to the tumor volume ;
- a frontal or occipital field depending on the tumor localization.

Absorbed doses of 2.50 Gy were delivered four times a week. A total dose of 55 Gy was delivered to the tumor volume ; the opposite hemisphere received a third of that dose.

Blood samples were taken by venipuncture prior to the first radiotherapy session (i.e. control value for each patient) and 24 h after 10, 20 and 30 Gy to the tumor volume. Blood culture and chromosome preparation were made according to classical techniques.

2. Results and discussion

As shown in table 1, the frequency of dicentric and ring chromosomes increased with the cumulated doses but, as expected, remained very low. Analysis of the distribution of the cells according to the frequency of dicentrics and rings showed that neither the Poisson method (Table 2) nor the Qdr method (Table 3) can be used to estimate the inhomogeneity of doses in cases of repeated local exposures.

b. Persistence of chromosome aberrations in an accidentally irradiated subject

1. Methodology

Peripheral blood samples were taken from an otherwise normal healthy man from 99 months up to 251 months (21 years) after an accident involving an nuclear excursion in December 1965. The irradiation was inhomogeneous and the estimated doses varied locally from 2 Gy to the head to 50 Gy to the left foot. Blood samples were cultivated at various intervals after the accident and 200 to 500 cells from each sample were examined for chromosome and chromatid aberrations.

2. Results and discussion

Confirming the previous reports, the presence of cells carrying a dicentric and its accompanying acentric fragment still detectable to date demonstrates that lymphocytes with induced chromosome aberrations can persist for many years in the peripheral blood (Table 4).

The fact that a few dicentrics were not accompanied by their expected fragment suggests, however, the possibility that some of them could be derived from aberrations produced in late stem cells. The yield of dicentrics showed a gradual decrease with time from 63 per 100 cells to 20 after 24 months then to 7.0 after 99 months and, finally, to less than 1.0 after 227 months. Due to the lack of relevant observations on the yield of aberrations soon after exposure, the present study does not allow us to resolve the question of the existence of an early plateau in the yield of aberrations which has been frequently observed during the first weeks after exposure. Neither does it provide additional information on the mean life span of that lymphocyte population that can be stimulated to divide with PHA. Our data, including the two earlier Fontenay points, fit relatively well to an exponential decrease and are in good agreement with the time effect relationship reported for dicentrics and centric rings in patients treated with X-irradiation for ankylosing spondylitis. Both sets of data can be fitted by the least squares method to a time hyperbolic curve $y = A = b\ t^{-1}$. The constant a was found to be 0.402 for the spondylitis and - 1.17 for our patient. The constants, b, were respectively 16.0 and 47.6 and the correlation coefficients 0.987 and 0.992. In projecting the curve for our patient over his normal life expectancy, the predicted yield of dicentrics and rings will have declined to background by about 40 years after the accident.

Our results together with those from A bomb survivors, patients treated with X rays for ankylosing spondylitis and Japanese fishermen exposed to fall-out radiation also suggest that the low and variable yield of dicentrics found after long intervals of time (more than 15 years) does not depend on the rate of aberrations produced initially.
In contrast to the changes in cells carrying unstable chromosome aberrations, the proportion of peripheral blood lymphocytes with translocations remained almost unchanged. Although no banded preparations were made in the present study, it could be clearly recognised that some cells showed identical translocations suggesting that they were derived from the same in vivo clone of stem cells.

Table 1 : Frequency and distribution of dicentric and ring chromosomes after irradiation

Case	Dose (Gy)	Cells scored	Total dicentrics and rings	Distribution of the cells according to the frequency of dicentrics and rings				
				0	1	2	3	4
1	0	200	0	200				
	10	77	0	77				
	20	200	1	199	1			
	30	200	0	200				
2	0	200	0	200				
	10	200	2	198	2			
	20	200	5	196	3	1		
	30	200	3	197	3			
3	0	200	0	200				
	10	200	0	200				
	20	200	0	200				
	30	200	8	194	4	2		
4	0	200	0	200				
	10	200	0	200				
	20	200	8	192	8			
	30	200	15	185	15			
5	0	200	3	197	3			
	10	200	1	199	1			
	20	200	6	194	6			
6	0	200	0	200				
	10	200	5	195	5			
	20	200	1	199	1			
	30	200	3	198	1	1		
7	0	200	0	200				
	10	200	2	198	2			
	20	200	1	199	1			
	30	200	7	195	3	2		
8	0	200	0	200				
	10	200	3	197	3			
	20	200	11	192	5	3		
	30	200	11	191	7	2		
9	0	200	1	199	1			
	10	200	1	199	1			
	20	114	6	196	3		1	
	30	200	13	190	8	1	1	
10	0	200	0					
	10	200	4	197	2	1		
	20	200	3	197	3			
	30	200	6	194	6			

Table 2 : Dose and irradiated fraction estimated on the basis of the in vitro dose-response curve

Dose administrated (Gy)	Dose estimated (Gy)	Irradiated fraction
10	1.33	0.10
20	2.15	0.14
30	3.09	0.17

Table 3 : Dose and irradiated fraction estimated by the Qdr-method

Dose administrated (Gy)	Dose estimated (Gy)	Irradiated fraction
10	0.71	0.81
20	2.49	0.49
30	2.62	0.21

Table 4 : Results of the cytogenetic observations performed in Mol

Time interval between irradiation & observation (months)	Cells analysed	Abnormal cells		Chromatid gap		Isochromatid gap	Chromatid break		Chromosome break		Acentric ring	Centric ring	Translocation		Dicentrics	
		Observed	Per 100 cells	Observed	Per 100 cells		Observed	Per 100 cells	Observed	Per 100 cells			Observed	Per 100 cells	Observed	Per 100 cells
99	500	42	8.4	8	1.6	1	1	0.2	19	3.8			16	3.2	35	7.0
107	500	52	10.4	13	2.6		3	0.6	25	5.0	2	2	15	3.0	34	6.8
174	500	26	5.2	5	1.0		5	1.0	1	0.2			11	2.2	8	1.8
180	400	16	4.0	4	1.0				2	0.5			12	3.0	3	0.8
189	200	12	6.0	3	1.5		2	1.0	5	2.5		1	4	2.0	3	1.5
195	400	27	6.75	14	3.5		1	0.2					9	2.2	5	1.2
227	500	13	2.6	4	0.8				2	0.4			5	1.0	4	0.8
245	500	16	3.2										15	3.0	1	0.2
251	500	10	2.0	1	0.2								9	1.8		

IV Objectives for the next reporting period

V Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. A. Wambersie, Radiobiology and Radiation Protection Unit, Catholic University of Louvain, B-1200 Brussels

Prof. M. Lemaire, Radiation Therapy Unit, University of Liège, B-4020 Liège, Belgium

VI. Publications:

L. Fabry, A. Léonard, G. Decat, Gh. Deknudt, P. Jacquet, E.D. Léonard : Chromosome aberrations in mixed cultures of in vitro irradiated and unirradiated human lymphocytes. Strahlentherapie und Onkologie 164 (1988) 108-110.

A. Léonard, Gh. Deknudt, E.D. Léonard : Persistence of chromosome aberrations in an accidentally irradiated subject. Radiation Protection dosimetry 22 (1988) 55-57.

Title of the project no.: 2

Accurate estimation of dose effect relationship for chromosome aberrations induced in human lymphocytes at low doses of X-rays.

Head(s) of project:

Alain LEONARD

Scientific staff:

Ghislain DEKNUDT

I. Objectives of the project:

To irradiate blood in vitro to low doses of X-rays and to examine the lymphocytes in metaphase for radiation induced chromosome aberrations. The primary objective is to verify the existence of any low dose plateau in response over the range zero to a few tens of milligrays. Blood from 20 donors will be used because variations in sensitivity of donors may influence the low dose response. All cells containing exchange type aberrations will be photographed and karyotyped in order to determine whether certain chromosomes are specifically involved in such aberrations.

II. Objectives for the reporting period:

1. To complete scoring the material at 0 and 30 mGy
2. To decode and collate the results from the 6 collaborating laboratories
3. To analyse the data for :
   a) interlaboratory variation
   b) comparison of yields at 0 and 30 mGy
   c) possible donor variability
   d) multiply damaged cells
   e) karyotyping cells with exchange aberrations

III Progress achieved

Methodology

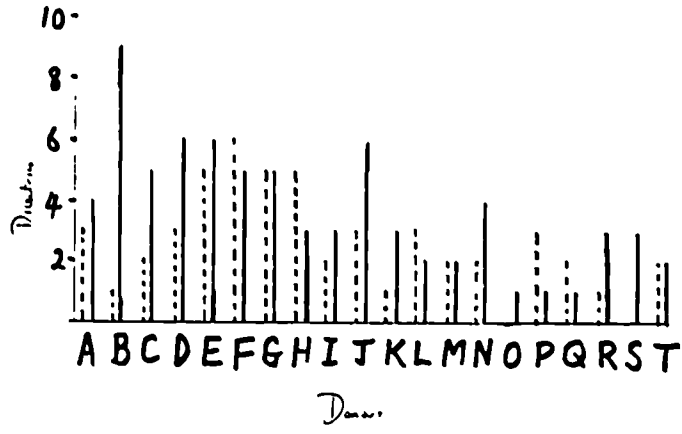
Standard differnece tests were applied to the data to determine the objectives a-c in section 2.

Results

Lab.	Dose (mGy)	No. Cells scored	Dicentrics	C. Rings	Excess Acentrics
1	0	10500	14	1	63
	30	10200	3	3	66
2	0	10000	9	0	2
	30	10000	11	1	8
3	0	10015	10	0	28
	30	9980	23	2	39
4	0	10000	5	0	16
	30	10000	11	1	17
5	0	9797	8	0	16
	30	9718	16	0	34
6	0	10000	5	0	9
	30	10000	10	0	26
Total	0	60312	51	1	134
	30	59898	74	7	190

The pooled donor results from the 6 laboratories.

Fig.



The pooled laboratory results for dicentric aberrations in the 20 donors. For each donor 3,000 cells were scored for zero dose (broken lines) and 3,000 cells for 30 mGy (solid lines).

Discussion

As is usually experienced in collaborative projects some inter-laboratory differences in results are apparent. This is especially evident for the acentric aberrations reported by laboratories 1 and 2. We have found no particular explanation for this divergence. The total numbers of dicentrics scored by each laboratory are reasonably consistent but laboratory 1 is exceptional in having found significantly more dicentrics in the controls than in the irradiated cells. This is mainly due to the very low yield at 30 mGy but the zero dose yield is a little high. The latter includes one cell containing 3 dicentrics from donor F (see later).

For acentric aberrations the inter-laboratory variations tend to cancel out and the total results are approximately consistent with a linear extrapolation of published dose response data. For dicentrics the background yield is consistent with previous reports of approximately 1 per 1000 cells. The yield in the irradiated cells however is low when compared with the data reported by this group of collaborating laboratories in a previous experiment (see section 6) and by extrapolating other published higher dose data assuming the linear quadratic model. Even if one excludes the odd result from laboratory 1 the yield is still low but tests show that the significance is marginal. These data therefore tend to support the idea of a threshold or plateau effect in the very low dose response.

A test on the distributions of the control and exposed sets of dicentric data shown in the Figure indicated no significant deviation from the Poisson distribution (zero dose $\sigma^2/y = 1.03 \pm 0.3$, 30 mGy $\sigma^2/y = 1.13 \pm 0.3$). This suggests that if inhomogeneity exists within this group of 20 subjects it could only be detected by many more cells being scored. There is a suggestion from the data in the Figure that subjects with a low control yield also exhibited a low irradiated yield and conversely a high control is associated with a high irradiated yield. However this trend is not quite significant but will be subject to further testing when data from two more doses become available.

Three multiply damaged (> 1 dicentric) control cells were observed ; one in each of donors F, H and J, and one irradiated cell in donor N. This is a similar finding to the previous experiment (section 6) and is again significantly greater than expected from Poisson statistics.

Karyotyping the cells containing exchange aberrations is still in progress but no result is yet available.

Because of the anomalies and variations described above it was resolved to score the other cells irradiated at 5 and 300 mGy that were prepared at the same time. The slides have been distributed to the collaborating laboratories and scoring is in progress.

IV Objectives for the next reporting period

To complete scoring the material irradiated at 5 and 300 mGy. To collate those results with the zero and 30 mGy data reported here. To analyse the full data in accordance with the objectives as described in sections 1 and 2 and to compare the results with those of a previous experiment now published (section 6).

V Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. State University of Leiden, The Netherlands (Prof. A. Natarajan)
2. Free University of Berlin, Germany (Prof. G. Obe) (since transferred to University of Essen)
3. CEN/SCK, Mol, Belgium (Dr A. Léonard)
4. BNFL, Sellafield, UK (Dr J. Tawn)
5. University of Rome, Italy (Dr F. Palitti)

VI. Publications:

D.C. Lloyd, A.A. Edwards, A. Léonard, G.L. Deknudt, A. Natarajan, G. Obe, F. Palitti, C. Tanzarella, E.J. Tawn : Frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of X-rays. Int. J. Radiat. Biol., 53 (1), 49-55, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-E-166-NL

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. P.H.M. Lohman
Dept. Rad. Genetics & Chem. Mutag.
State University of Leiden
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NL - 2333 AL Leiden

Telephone number: 071-148333-6175

Title of the research contract:

Radiation sensitivity in cultured mammalian cells, the genetic effects of radiation in eukaryotes and chromosome aberrations in human lymphocytes.

List of projects:

1. Isolation and characterization of DNA repair genes.
2. Biochemical analysis of DNA repair.
3. DNA repair and mutagenesis.
4. The relationship between DNA repair processes and the nature and magnitude of genetic damage induced by X-irradiation.
5. Studies on mutations and their repair in Drosophila.
6. Evaluation of the frequencies of chromosomal aberrations induced in human blood by low doses of X-rays (1-10 rad).

Title of the project no.: 1

Isolation and characterization of DNA repair genes.

Head(s) of project: Dr. J.W.I.M. Simons.

Scientific staff: Dr. M.Z. Zdzienicka.

I. Objectives of the project:

This project aims to isolate and characterize repair deficient mutants from mammalian cell cultures. Via replica plating clones are isolated which are sensitive to DNA-damaging agents. In the first instance rodent cells are used (V79 Chinese hamster cells and CHO cells).
Isolated repair deficient mutants will be characterized in terms of survival after treatment with a variety of DNA-damaging agents. Via complementation analysis it will be ascertained whether they belong to different omplementation groups and whether they complement repair deficient mutants isolated by other laboratories. Also, the complementing ability with known human repair deficient syndromes will be investigated.

II. Objectives for the reporting period:

1. Further isolation and characterization of repair deficient mutants.
2. Search for a methodology for the isolation of mutagen sensitive cells from a human cell line.

III. Progress achieved:

Isolation and characterization of repair deficient mutants (including isolation of mutagen sensitive cells from a human cell line).

Methodology.

The methodologies have been described extensively in the 1986 report.

Results.

The three x-ray sensitive mutants V-C4, V-E5 and V-G8 which belong to one complementation group show also increased sensitivities to bleomycin (3.5 fold) and ethyl methane sulfonate (2-fold). Some difference in the phenotypes exist also as V-C4 has a more than 2-fold enhanced sensitivity to UV, while V-G8 has a normal sensitivity. The mutants have a normal repair of single and double-strand breaks. Like cell lines derived from patients with Ataxia telangiectasia these mutants have an impairment of DNA synthesis inhibition after X-ray irradiation. They (except V-G8) also have an enhanced level of spontaneously occurring chromosome aberrations and all have after X-irradiation a 3-4 fold higher level of chromatid gaps, breaks and exchanges than wild type cells. In addition, G1-irradiation of the mutants yielded both chromosome and chromatid types of aberrations. Thus both the level and pattern of chromosomal aberrations induced by X-rays are similar to those observed in AT cells.

These results indicate that our mutants represent the first rodent cell mutants which show phenotypic characteristics of AT cells.

Experiments have been started which aim to develop a methodology for the isolation of repair deficient cells from a human cell line. As basis for selection has been chosen the application of viral suicide: Herpes simplex virus I is treated with a mutagen; repair deficient cells would not be able to remove the damage from the viral DNA and in this way escape cell lysis. Factors to be taken into account are : multiplicity of infection, dose of mutagen, number of cells per petri dish, treatment as single cells or as clones, duration of infection, prevention of spread of infection, cure of cells with an ongoing infection etc.

The results obtained sofar are that conditions have been found for UV-irradiated virus in which wild type cells are wiped out while cells from patients with Xeroderma pigmentosum group A completely survive the treatment. These experiments have been performed in cooperation with Dr. Abrahams from Leiden.

Discussion.

As quite a number of repair-deficient mutants are now available and genetically characterized, further research will put no emphasis on this point exept for the development of a methodology for isolation of repair deficient human cells.

A new aim within this project will be the isolation of a human gene which is able to correct one of our repair deficient mutants. Therefore experiments will be started on the transfectability of our cells to assess the probability of isolation of transfectants.

IV. Objectives for the next reporting period:

1. Continuation of complementation analysis of our repair deficient mutants.
2. Development of a viral suicide method for the isolation of human repair deficient cells.
3. Experiments on the transfectabiltity of the repair deficient rodent cell lines.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Department of Cell Biology and Genetics, Rotterdam (Prof. Dr. D. Bootsma)
MRC Cell Mutation Unit, Brighton (Prof. Dr. B.A. Bridges)
Department of Biochemistry, Leiden (Prof. Dr. P. van de Putte)
Department of Molecular Carcinogenesis, Leiden (prof. Dr. A. van de Eb)
Medical Biological Laboratory, Rijswijk (Dr. R.A. Baan)
Anthropogenetisch Instituut, GU Amsterdam (Dr. F. Arwert)

VI. Publications:

Zdzienicka, M.Z., G.P. van der Schans, A. Westerveld, A.A. van Zeeland and J.W.I.M. Simons. Phenotypic heterogeneity within the first complementation group of UV sensitive mutants of Chinese hamster cell lines. Mutation Res. 193 31-41 1988.

Zdzienicka, M.Z., G.P. van der Schans and J.W.I.M. Simons. Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster cells. Mutation Res. 194 165-170 1988

Zdzienicka, M.Z., J.W.I.M. Simons and P.H.M. Lohman. Chinese hamster cell lines defective in DNA repair. In: DNA repair mechanisms and their biological implications in mammalian cells. Ed. M.W. Lambert. Plenum Press. N.Y. (in press).

Title of the project no.: 2

Biochemical analysis of DNA repair

Head(s) of project: Dr.Ir. A.A. van Zeeland
Dr. L.H.F. Mullenders

Scientific staff: Dr.Ir. A.A. van Zeeland
Dr. L.H.F. Mullenders
Dr. M.Z. Zdzienicka
Drs. J. Venema

I. Objectives of the project:

In this project we will study the biochemical aspects of DNA repair induced by radiation, in normal cells as well as radiosensitive mutants, in relation to biological endpoints such as cell killing, induction of gene mutations, and chromosomal aberrations. Radiosensitive cell lines in which the radiosensitivity is complemented by the introduction of a cloned repair gene will also be investigated. Emphasis will be put on: (a) the role of chromatin structure in the distribution and repair of damage induced by radiation, (b) the nuclear localization of the various steps of the repair process, and (c) the structure of DNA repair patches using inhibitors of DNA synthesis.

II. Objectives for the reporting period:

1. Repair synthesis in higher order loops.
2. The removal of UV-induced pyrimidine dimers in single copy genes.
3. Establishment and chracterization of a permeable cell system.
4. Biochemical characterization of X-ray-sensitive mutants (V-15B, VC-4, VE-5 and V-G8) derived from V79 Chinese hamster cells.

III. Progress achieved:

Repair synthesis in higher order loops.

Methodology

The distribution of repaired sites in higher-order chromatin loops has been analyzed in stationary human fibroblasts. Repair synthesis was detected by incorporation of ^{3}H-TdR in the presence of hydroxyurea, which allows measurement of repair by effective and specific inhibition of normal replicative synthesis. The distribution of repaired sites was investigated by digestion of DNA-nuclear matrix complexes with DNAse 1.

Results and discussion

The eukaryotic DNA is organized in supercoiled loops by anchorage to a protein structure termed nuclear matrix (interphase) or scaffold (mitosis). Topoisomerase II has been identified as an integral component of both structures and this finding has led to the suggestion that the nuclear matrix somehow poise chromatin domains for transcription by allowing torsonial stress to be introduced into defined regions. Nonrandom organization of chromatin is further suggested by the presence of specific DNA sequences including regulatory sequences at the base of loops associated with the nuclear matrix. Such an organization is thought to bring about functional compartimentalization within the nucleus and facilitates DNA replication and transcription processes both occurring in close association with the nuclear matrix. We previously reported that in normal fibroblasts, DNA repair synthesis following a low dose of UV-irradiation (5 J/m^2) was preferentially found in nuclear matrix associated DNA. Further experiments revealed that this non-random distribution of repaired sites initially following irradiation, represented the preferential repair of DNA sequences permanently bound to the nuclear matrix. These results were obtained with a biochemical approach, that requires the use of confluent cells and the presence of inhibitor(s) during repair. We employed the fluorescence DNA-halo technique and autoradiography to investigate the role of the nuclear matrix in UV-induced repair at the single cell level in exponentially growing fibroblasts in the absence of inhibitors. Consistent with the results of the biochemical experiments we found a preferential repair of nuclear matrix associated DNA in normal fibroblasts UV-irradiated with 5 J/m^2.

So far experiments have been performed by high salt (2 M NaCl) extraction of nuclei to obtain histone-depleted DNA-nuclear matrix complexes. Conclusions with regard to the intranuclear localization of DNA repair are based on the assumption, that nuclear matrix associated repair complexes are refractory to the high salt isolation procedures. Since this assumption may not be correct, we analyzed the distribution of repaired sites in DNA-nuclear matrix complexes isolated at low ionic strength using the detergent lithium diiodosalicylate (LIS). We found that the distribution of repaired sites in DNA-nuclear matrix complexes isolated by either high or low salt extraction of nuclei, was essentially the same. Our results suggest that repair of UV-induced damage is not confined to the nuclear matrix as has been shown for replication and transcription. The efficiency of repair may be based on a sliding model as has been proposed for the incision of bulky damage by the uvr ABC enzyme of E. coli. Yet it is clear that in human fibroblasts certain domains within the chromatin are more rapidly repaired than the bulk of the genome. These domains are located proximal to the nuclear matrix, and comprise transcriptionally active DNA. We have previously shown that the heterogeneity in distribution of repaired sites correlate with heterogeneity in removal of pyrimidine dimers from the genome. However, it is possible that at time periods initially following irradiation repair of 6-4 photoproducts substantially

contributes to repair synthesis. In that case our data suggest that repair of 6-4 photoproducts is subject to the same regime as removal of pyrimidine dimers i.e. preferentially directed towards repair of transcriptionally active domains.
We have investigated the role of the transcription process in UV-induced repair. In a 18.5 kb restriction fragment of the human adenosin deaminase (ADA) gene, no differences were found in removal of pyrimidine dimers from the transcribed and non-transcribed strands. Both strands are repaired much faster than the genome overall. A cell line containing a small deletion in the promotor and therefore deficient in the synthesis of ADA m-RNA, still possessed the capacity to perform preferential repair of the ADA gene. These results suggest no direct involvement of the transcription process itself in preferential repair of active genes.

The removal of UV-induced pyrimidine dimers in single copy genes.
Methodology
Removal of UV-induced pyrimidine dimers from defined DNA fragments was investigated in primary human fibroblasts either exponentially growing or in a confluent state. Quantification of pyrimidine dimers present in restriction fragments of genes of interest was performed by digestion with the dimer-specific enzyme T4 endonuclease V, alkaline agarose gel electrophoresis, Southern transfer and hybridization with specific DNA probes. The induction and removal of dimers was measured in the transcriptionally active adenosine deaminase (ADA) and dihydrofolate reductase (DHFR) genes, and the inactive 754 locus. Experiments were performed with normal human and UV-sensitive human cell strains i.e. xeroderma pigmentosum group C and Cockayne's syndrome fibroblasts.
Results and discussion
In UV-irradiated hamster V79 cells we found a rapid and efficient removal of pyrimidine dimers from the HPRT gene, in contrast to the very slow and inefficient repair of the genome overall. The UV-sensitive derivative VH-1 was completely deficient in preferential repair of pyrimidine dimers from the HPRT gene. These differences in repair of the HPRT gene correlate well with the 10-fold increase of UV-induced HPRT mutations in VH-1 compared to V79 wild type cells. Experiments aimed to determine removal of pyrimidine dimers from the transcribed and non-transcribed strand of the HPRT gene in V79 cells are in progress.

Establishment and chracterization of a permeable cell system.
Methodology
Experiments aimed to establish a permeable cell system have been based on the procedures described by Dresler et al. (Biochemistry 21, 2557, 1982) with minor modifications in the preparation of cell suspensions from human fibroblasts. Briefly cells were forced through a 21 g. hyperdermic needle and permeabilized by hypertonic treatment. To assay the capacity of the system to perform repair synthesis UV-irradiated cells were permeabilized, labelled with ^{3}H-dCTP in the presence of bromodeoxyuridine triphosphate, and the DNA was purified in CsCl density gradients to obtain non-replicative DNA.
Results and discussion
In vitro UV-induced repair synthesis was performed by α-dCTP labelling of permeabilized cells. Only small levels of repair synthesis could be detected in stationary human fibroblasts permeabilized prior to UV-irradiation. However, repair synthesis was strongly enhanced when stationary cells were UV-irradiated prior to permeabilization or when the pyrimidine dimer specific enzyme T4 endonuclease V was introduced into the permeable cells. Excision repair deficient xeroderma pigmentosum group A

cells were not capable to perform repair synthesis, unless T4 endonuclease V was added to the cells. In preliminary experiments we were not able to correct the repair deficient phenotype of XP-A cells by adding cellular extract of normal human fibroblasts, possibly due to inappropriate concentration of the extract.

Biochemical characterization of X-ray-sensitive mutants (V-15B, VC- 4, VE-5 and V-G8) derived from V79 Chinese hamster cells.

Methodology

Accumulation of single strans breaks (SSB) in the presence of HU and ara-C and the rate of their rejoining was measured by alkaline elution. The rejoining of double strand breaks (DSB) was measured via neutral elution. Inhibition of DNA synthesis was measured via the uptake of labeled thymidine.

Results and discussion

The mutant V-15B is about 8-fold more sensitive to X-ray as judged by D10 values and belongs to the same complementation group as the xrs mutants described by Jeggo. Biochemical characterization showed that this mutant has a decreased ability to rejoin X-ray induced double styrand breaks. After 4 hours of repair more than 50% of the double-strand breaks remain in comparison to 3% in wild type cells. No difference was obseved in the initial number of single strand breaks induced, in the kinetics of their rejoining and in the final level of unrejoined single-strand breaks.

The mutants V-E5, V-C4 and V-G8, which belong to a new complementation group show a normal initial number of induced single and double strand breaks, normal kinetics of their rejoining and a normal final level of unrejoined single and double strand breaks.

The dose-response curves of the inhibition of DNA synthesis showed that V-E5, V-C4 and V-G8 have an diminished inhibition of DNA synthesis compared to wild type V79 cells. After exposure to 20 Gy, the residual rate of DNA synthesis was about 40% for wild type cells while it was about 80% for the X-ray sensitive mutants.

The biochemical characterization of the new complementation group indicates a similarity to the phenotype of cells derived from patients with Ataxia telangiectasia.

IV. Objectives for the next reporting period:

We will continue to study (1) the removal of UV-damage from defined DNA fragments of active and inactive genes, with special emphasis to the role of the transcription process itself in DNA repair. The effect of sodium butyrate (enhanced repair, but no survival recovery) on gene specific repair will be included in these studies as well. (2) Further characterization of the in vitro repair system will be performed by using either concentrated extracts or partially purified proteins (in collaboration with Prof. D. Bootsma, Erasmus University, Rotterdam, The Netherlands). (3) The relationship between preferential repair of nuclear matrix associated DNA and localization of genes relative to the nuclear matrix will be investigated. (4) Biochemical characterization of repair deficient mutants with respect to the removal of adducts induced by the radiomimetic agent 4-NQO.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. L. Mayne, Sussex, U.K.
Dr. A. Smith, Stanford University, U.S.A.
Dr. P. Dijkwel, Virginia State University, U.S.A.

VI. Publications:

Mullenders, L.H.F., A.C. van Kesteren-van Leeuwen, A.A. van Zeeland and A.T. Natarajan. Nuclear matrix associated DNA is preferentially repaired in normal human fibroblasts, exposed to a low dose of UV light, but not in Cockayne's syndrome fibroblasts. Nucl. Acids Res. 22, 10607-10622 (1988)

Mayne, L.V., L.H.F. Mullenders and A.A. van Zeeland. Cockayne's syndrome: a UV-sensitive disorder with a defect in the repair of transcribing DNA but normal overal excision repair. In 'Mechanisms and consequences of DNA damage processing', in press.

Mullenders, L.H.F., J. Venema, L. Mayne, A.T. Natarajan and A.A. van Zeeland. Non random distribution of UV-induced repair in higher-order chromatin loops in human cells and its relationship to preferential repair of active genes. In 'DNA repair mechanisms and their biological implications in mammalian cells', in press.

Zdzienicka, M.Z., Q. Tran, G.P. van der Schans and J.W.I.M. Simons. Characterization of an X-ray-hypersensitive mutant of V79 Chinese hamster cells. Mutation Res. 194 239-249 1988.

Mitchell, D.L., M.Z. Zdzienicka, A.A. van Zeeland and R.S. Nairn. Intermediate (6-4) photoproduct repair in Chinese hamster V79 V-H1 correlates with intermediate levels of DNA incision and repair replication. Mutation Res. (in press).

Title of the project no.: 3

DNA repair and mutagenesis.

Head(s) of project: Prof. Dr. A.T. Natarajan
Dr. J.W.I.M. Simons

Scientific staff: Dr. M.Z. Zdzienicka
Drs. F. Darroudi
Dr. H. Vrieling

I. Objectives of the project:

To study the mechanisms involved in the induction of gene mutations and chromosomal aberrations by influencing the DNA repair pathways. Several methods are used to alter the repair pathways: use of repair-deficient cells, use of DNA-repair inhibitors and mutagenic treatment with X-irradiation, UV or radiomimetic chemicals.

II. Objectives for the reporting period:

1. Characterization of the X-ray sensitive mutants V15-B, V-G8, V-C4 and V-E5.
2. Determination of the fidelity of DNA replication after mutagenic treatment.
3. Cytogenetic characterization of X-ray sensitive Chinese hamster ovary (CHO) cell mutants.

III. Progress achieved:

Characterization of the x-ray sensitive mutants V15-B, V-G8, V-C4 and V-E5.

Methodology

a. Determination of mutation induction.

Because the mutant V15-B can tolerate only low doses of X-rays and as X-rays are not very efficient in the induction of mutations, a protocol had to be used which lowers the background of pre-existing mutants: 250 cells were grown to a population of about 20-40X10^6 cells for each experiment. This population was subsequently split into two parts: one part was used as a control and the other part was X-irradiated.

b. Analysis of chromosomal aberrations.

Cells synchronized by mitotic shake off were used for the irradiation in G1. Mitotic cells were plated and after 90 minutes irradiated with 1 Gy X-rays. The cells were fixed 18 hours after irradiation. For G2 studies, exponentially growing cells were irradiated with 0.3 Gy and allowed to recover for 2 hours before colcemid was added. Giemsa stained slides were scored for the presence of chromosomal aberations, 50 to 200 cells were scored per point.

Results

a. Determination of mutation induction in V15-B after X-rays.

Despite that V15-B cells are approximately 8-fold more sensitive to X-rays than wild type cells the mutation induction was not significantly enhanced. The muation induction at the HPRT locus was 0.9X10^{-5} per Gy for control cells whereas it was 1.7X10^{-5} for V15-B.

b. Analysis of chromosomal aberations.

Despite the fact that V-G8, V-C4 and V-E5 belong to the same complementation group, different levels of spontaneous chromosomal aberrations were observed. For V-G8 these frequencies were similar to that observed in wild type cells whereas an increase of about 2- and 6-fold was found for V-E5 and V-C4 respectively. In all three mutants the frequencies of x-ray induced aberrations were higher in comparison to wild type V79 cells in both G1 and G2 cells. The type of aberrations induced in G1 cells were mainly of chromosome type in the normal cells, whereas in all three X-ray sensitive mutants both chromosome and chromatid exchanges were found and occurred within the same cell.

Discussion

The enhanced level of chromosome aberrations after X-ray irradiation in V-G8, V-C4 and V-E5 and the enhanced spontaneous chromosomal instability is similar to that observed in AT cells. Also the occurrence of both chromatid and chromosome types of aberrations following irradiation in G1 is similar to the observations made in cells from patients with Ataxia telangiectasia. Therefore this new complementation group of X-ray sensitive mutants appears phenotypically similar to AT.

Determination of the fidelity of DNA replication after mutagenic treatment.

Methodology

GRSL mouse lymphoma cells are treated with a mutagen and seeded in subpopulations of 100 viable cells each. Each subpopulation is grown to 2X10^6 cells and the mutant frequency per culture is determined. This procedure allows to discriminate between directly induced mutations, which lead to large numbers of mutant clones in a few of the subpopulations and delayed mutations, which, if present, will lead to much smaller numbers of mutant colonies.

Sequence analysis of HPRT mutants. Mutant RNA was used as a template in

PCR. The amplified cDNA was ligated in a M13 vector, the HPRT-cDNA was identified in plaques and sequenced. Per mutant two cDNA's were sequenced to verify the observed mutation.

Results

a. Induction of a SOS-like response.

As treatment with ENU leads to infidelity of DNA replication (see previous report) the response after ENU has been studied in greater detail. Mutation spectra have been determined from directly induced mutations and from delayed mutations. The mutation spectrum of the directly induced mutations agrees with published data on ENU-induced spectra (60% GC to AT transitions, 10% AT to GC transitions and 30% transversions). The mutation spectrum of delayed mutations is very different and consists for over 90% out of transversions. Moreover the delayed mutational response generates only mutations in one strand and at AT-base pairs. The number of populations with a delayed mutation increases from generation 5 to 7 after treatment and the delayed response has disappeared at the 12th generation after treatment. Because of these kinetics of the response it seems that a SOS-like response has been induced by the mutagenic treatment.

b. Induction of infidelity of DNA replication by UV and X-rays.

The experiments with UV led to a significant induction of delayed mutations. This effect could only be observed when all experiments were pooled. Therefore the induction of an indirect mutational response is much less after UV than after ENU.

Three experiments have been performed after irradiation with X-rays. Although there is some evidence for the induction of infidelity of DNA replication the difference is not significant.

Discussion

The indication for a SOS-like response in mammalian cells raises many questions. The first is the generality of the response. The resolving power of the fluctuation assay appears still not adequate enough for all mutagenic treatments. A further increase in the number of subpopulations of the fluctuation assay is indicated. Presently the number of subpopulations which can be handled in a single experiment is about 150. If the number of populations could be raised to about 500 it would be possible to register a temporarily increase of the mutation rate from $3X10^{-7}$ to 10^{-6}.

Cytogenetic characterization of X-ray sensitive Chinese hamster ovary (CHO) cell mutants.

Xrs 5 and Xrs 6 mutants (isolated by Dr. Jeggo) are defective in repair of DNA double strand breaks (DSBs). The frequencies of X-ray induced aberrations are many fold higher in these mutants in comparison to wild type CHO cells. Further characterization was made by following experiments.

Methodology

Chromosomal aberrations, SCEs and cell survival were analyzed in the two mutants and the wild type cells after treatment of synchronized or exponentially growing cells with bleomycin, (inducer of strand breaks) simple monofunctional alkylating agents (inducer of base damage), bifunctional alkylating agents (inducer of cross links) restriction endonucleases (inducer of DNA DSBs), inhibitors of topoisomerase II (inducer of DSBs and SSBs). In addition experiments were done to evaluate the influence of oxygen on the radiation induced chromosomal aberrations by irradiating under aerobic and anaerobic conditions.

In order to check whether the increased damage in the mutant cells can be rectified by the wild type cells, premature chromosome condensation technique (PCC) was employed. The following fusions were made using poly ethylene glycol:

X-irradiated mutant cells + mitotic wild type cells
X-irradiated wild type cells + mitotic mutant cells
X-irradiated mutant cells (xrs 5) + mitotic mutant cells (xrs 6).
The induction and repair of SSBs were measured by using a novel single strand specific antibody (in collaboration with van der Schans, MBL, Rijswijk).
Survival and frequency of mutations induced were determined by growing cells following different doses of UV or X-rays in the presence or absence selective agents, namely 6-thioguanine (HPRT locus) or oubaine (Na+/K+ ATPase) and counting viable colonies.

Results and Discussion

The results of the studies designed to evaluate the frequencies of induced chromosomal aberrations, sister chromatid exchanges (SCEs) and cell killing in CHO-K1, xrs 5 and xrs 6 cells after treatment with a series of chemicals with different modes of action are summarized in Table 1. The data indicate that there is a link between defect in DNA double strand breaks (DSBs) repair and increased biological effects (i.e. cell killing and chromosomal aberrations) after treatment with bleomycin and alkylating agents. However, the responses of the xrs mutants to all chemical mutagens and UV (acting S-dependently) for induction of SCEs was at the level of wild-type cells. Thus the lesions or pathways involved in SCEs production at least in part are different from those responsible for cell killing and/or chromosomal aberrations. Further evidences for the involvement of DNA DSBs in the formation of chromosomal alterrations obtained when restriction endonucleases (inducing only one class of lesion, DSB), and inhibitors of DNA topoisomerase II (m-AMSA and VP 16) were employed (Table 1). These agents act like X-rays and higher sensitivity of xrs 5 and xrs 6 cells for chromosomal aberrations and SCEs was evident when compared to CHO-K1 cells. The results indicate that DNA DSBs induced by restriction endonucleases and inhibitors of topoisomerase II correlate closely with chromosomal aberrations and SCEs in these cell lines, indicating DSBs to be responsible for the production of these two genetic end points.
X-rays induce a variety of lesions in the cellular DNA, such as DSBs, single strand breaks (SSBs), base damage (BD) and DNA-protein cross links. The data with xrs mutants defective in DNA DSBs repair clearly indicate involvement of DSBs induced with X-rays for the formation of chromosomal aberrations (chromosome-type aberrations in G_1 and chromatid-type aberrations in G_2). However X-ray or bleomycin treatment of G_1-cells led to increase in the frequencies both chromosome and chromatid type aberrations in xrs 5 and xrs 6 cells in comparison to the induced frequency of only chromosome type aberrations in CHO-K1 cells. In addition, the frequency of SCEs increased in xrs 6 cells following treatment with X-rays or bleomycin (Table 1). Therefore, it is assumed that xrs mutants could be also deficient in rejoining of other lesions, i.e. SSB and/or BD which persist in DNA and reach to the S-phase and misrepaired.
The rejoining of DNA SSBs induced by τ-rays was studied in these cell lines by a newly developed immunochemical method. This method is based on the binding of a monoclonal antibody to single-stranded DNA. No difference was detected between wild-type and mutant strains in the initial number of SSBs induced. The kinetics of repair were also similar between wild-type and xrs mutant CHO cells (Fig. 1). A fast removal followed by a much slower decrease, and the amount of SSB rejoining reach after 2 and 4 hours to the same level in all cell lines. To study the ability of xrs mutants to repair BD induced by X-rays, the cells were X-irradiated in air (to increase the ratio between DSB to BD) and in nitrogen (to increase the ratio between BD to DSB), and frequencies of chromosomal aberations and SCEs were studied. The xrs mutants defective in repair of DNA DSBs exhibit

a markedly enhanced sensitivity to aerobic irradiation, and high level of oxygen enhancement ratio (OER), 1.6-2-fold higher than CHO-K1 cells (Fig. 2). The induction of SCEs was comparatively not affected in CHO-K1 and xrs 5 (OER=1), in contrast dose dependent increase in the frequency of SCEs was obtained in xrs 6 cells treated with X-rays, and further increase by a factor of 2 was evident in hypoxic condition (OER=0.4)(Fig. 3). These experimental evidences indicate that deficiency in DNA DSBs repair led to increase in the frequency of chromosomal aberrations. Base damage induced by X-rays could lead to the formation of SCEs and xrs 6 cells appear not to be able to repair completely this type of lesion.

PCC method has been used to study the complementation analysis of X-irradiated wild-type and xrs mutants. CHO cells were synchronized and X-irradiated in G_1-stage. G_1-interphase cells of CHO-K1, xrs 5 and xrs 6 were fused with mitotic cells of its own or each other, that induce the former to condense their chromosomes prematurely. The results revealed that CHO-K1 is capable of complementing the X-ray sensitivity in both xrs 5 and xrs 6 cells, which indicates the recessive nature of mutations, however, it could never fully rectify the defect (Fig. 4). This is probably because although the hybrids contain approximately twice as much DNA as diploid cells, they only contain one non-mutated copy of the appropriate DNA repair gene. Xrs 5 or xrs 6 cells were not able to complement the defect in each other, which indicates both belong to the same complementation group.

The correlation between X-ray and UV-induced cytotoxicity and induction of mutations at HPRT and Na^+/K^+-ATPase (Ouabain loci) in wild-type and xrs mutants was studied (Table 1). The high cell killing effect of X-rays in xrs mutants in comparison to wild-type cells (6-fold) was accompained with 2-3-fold higher mutability at HPRT locus. The xrs mutants were also more sensitive to cell killing effect of UV (1.5-fold), which correlate well with induced frequencies of TG^r and Oua^r mutants in xrs mutant cells, 2-3-fold higher than in CHO-K1 cells.

Table1:

COMPARATIVE STUDIES OF TWO X-RAY-SENSITIVE MUTANTS, XRS 5 AND XRS 6 WITH WILD-TYPE CHO-K1 CELLS

CELL TYPE	AGENT	SURVIVAL (D_{37})	MUTATION		CHROMOSOMAL ABERRATIONS			SCEs
			TG^r	Oua^r	Exponentially (G_1-S)	G_1	G_2	
xrs 5	spont.		3	1	1	1	1	1
xrs 6	spont.		4	3	1	1	1	1
xrs 5	X-rays	6	3	1*		12	7	1.5**
xrs 6	X-rays	6	2.5	1*		12	3.5	2.5**
xrs 5	UV	1.5	2	2		1.5		0.75
xrs 6	UV	1.5	3	3		2		1
xrs 5	Bleomycin	3				3		1.0
xrs 6	Bleomycin	13				4.5		2***
xrs 5	MMS	2.5	2	1.5	2		4 to 7****	0.8
xrs 6	MMS	2.5	2	1.5	1.5		2 to 5****	1
xrs 5	EMS	2			3.5			1
xrs 6	EMS	1.7			2.0			1
xrs 5	MMC	2.5				2		1
xrs 6	MMC	2				2		1
xrs 5	DEB	1.7				2		1
xrs 6	DEB	1.7				2		1
xrs 5	Res. enzymes				1.5-2			2
xrs 6	Res. enzymes				1.5-2			2
xrs 5	m-AMSA					3	20	2
xrs 6	m-AMSA					3	10	2
xrs 5	VP 16						4	
xrs 6	VP 16						2	

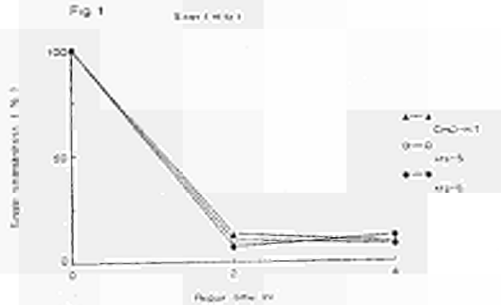
*) X-rays were not able to increase the frequency of mutation at Oua locus in any of these CHO cell lines.

**)The comparison is based on the X-ray dose of 1.5 Gy.

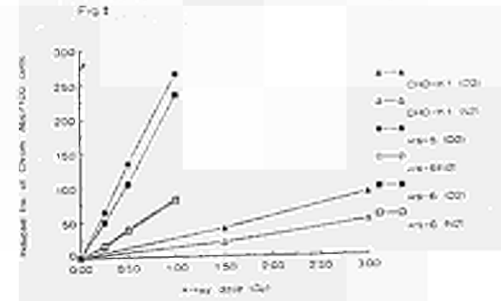
***) The comparison is based on the dose of 1.2 ug/ml.

****)The induced frquency of chromsomal aberrations compared to the spontaneous frequency of breaks in xrs mutant cell lines.

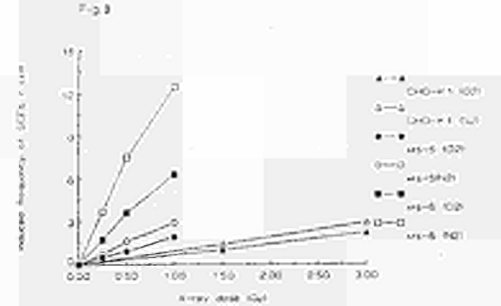
Abbreviations: UV, ultraviolet; MMS, methylmethane sulphonate; EMS, ethylmethane sulphonate; MMC, mitomycin C; DEB, diepoxy butane; Res. enzymes, restriction enzymes (Cfo I, EcoR I, Hpa II; Hae III, Alu I); m-AMSA, 4' -(9-acridinylamino)-methanesulfon-m-anisidide; VP 16, etoposid.



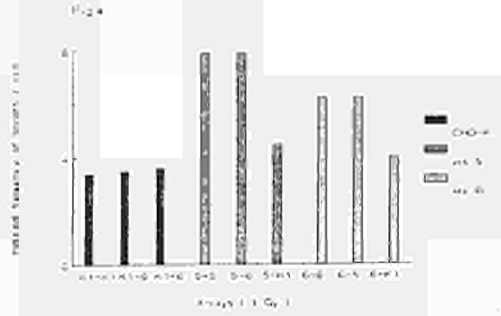
Kinetics of repair of SSBs after gamma-irradiation (10 Gy)



Frequencies of chromosomal aberrations in xrs5, xrs6 and CHO-K1 after X-irradiation in O_2 and N_2 atmosphere



Frequencies of SCEs on xrs5, xrs6 and CHO-K1 cells, following X-irradiation in N_2 and O_2 atmosphere



Frequencies of chromosomal breaks induced by 1 Gy X-rays in xrs mutants following fusion with CHO-K1 (vice versa) as determined in PCCS

IV. Objectives for the next reporting period:

1. Determination of mutation induction in repair deficient cells.
2. Experiments aimed at improving the resolving power of the fluctuation assay with mouse lymphoma cells.
3. Determination of the effect of ionizing radiation on the fidelity of DNA replication.
4. Influence of high LET radiations on the xrs-mutants (CHO) and V79 mutants.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Department of Cell Biology and Genetics, Rotterdam (Prof.Dr. D. Bootsma).
MRC Cell Mutation Unit, Brighton (Prof.Dr. B.A. Bridges).
Department of Biochemistry, Leiden (Prof.Dr. P. van de Putte).
Department of Medical Biochemistry, Leiden (Prof.Dr. L. van de Eb).
Medical Biological Laboratory, Rijswijk (Dr. R.A. Baan).
Inst. of Genetics, Free University, W. Berlin, F.R.G. (Prof. G. Obe).

VI. Publications:

Zdzienicka, M.Z., Q. Tran, G.P. van der Schans and J.W.I.M. Simons. Characterization of an X-ray-hypersensitive mutant of V79 Chinese hamster cells. Mutation Res. 194 239-249 1988.

Zdzienicka, M.Z., N.G.J. Jaspers, G.P. van der Schans, A.T. Natarajan and J.W.I.M. Simons. Ataxia-telangiectasia-like Chinese hamster V79 cell mutants with radioresistant DNA synthesis, Chromosomal instability and normal DNA strand break repair. Cancer Res. (in press).

Natarajan, A.T., J.M.J.J. Vossen and M.H. van Weel-Sipman. Aplastic Anemia and Fanconi Anemia: Response of Lymphocytes to X-rays and Mitomycin C. In Fanconi Anemia; Eds. T. Schroeder et al, Springer Verlag, 1989. pp. 100-104.

Lehman, A.R., Arlett, C.F., Broughton B.C., Harcourt, S.A., Steingrimsdottir, Steffani, M. Taylor, A. M. R., Natarajan,A.T., Green,S., King, M.D., Mackie, M., Stephenson, J.B., and Tolmie, J.N.: Trichothiodystrophy, a human DNA repair disorder with heterogenity in the cellular response to ultra violet light. Cancer Res. 48, 6090-6096, 1988.

Darroudi, F., A.T. Natarajan and P.H.M. Lohman (1988) Cytogenetical characterization of UV sensitive repair deficient CHO cell line 43-3B. II. Induction of cell killing, chromosomal aberrations and sister-chromatid exchanges by 4NQO, mono- and bifunctional alkylating agents, Mutation Res., in press.

Darroudi, F., A. Westerveld and A.T. Natarajan (1988) Cytogenetical characterization of Chinese hamster 43-3B transformanbts with the amplified or nonamplified human DNA repair gene ERCC-1, Mutation Res., in press.

Darroudi, F., and A.T. Natarajan (1988) Cytogenetical characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. III. Induction of cell killing, chromosomal aberrations and sister-chromatid exchanges by bleomycin, mono- and bifunctional alkylating agents, Mutation Res., in press.

Darroudi, F., and A.T. Natarajan (1989) Cytogenetical characterization of Chinese hamster ovary X-ray-sensitive mutant xrs 5 and xrs 6 cells. IV. Study of chromosomal aberations and sister-chromatid exchanges by restriction endonucleases and inhibitors of topoisomerase II, Mutation Res., submitted

Darroudi, F., A.T. Natarajan, G.P. van der Schans and A.A.W.M. van Loon (1989) Molecular and cytogenetical characterization of X-ray-sensitive Chinese hamster ovary mutant cells, xrs 5 and xrs 6. V. The correlation between DNA strand breaks and base damage to chromosomal aberrations and sister-chromatid exchanges induced by X-irradiation, Mutation Res., submitted.

Title of the project no.: 4

The relationship between DNA repair processes and the nature and magnitude of genetic damage induced by X-irradiation

Head(s) of project: **Prof. A.T. Natarajan**

Scientific staff:

Dr. A.D. Tates
Dr. P.P.W. van Buul
Drs. F. Darroudi
Prof. K. Sankaranarayanan
Dr. L.H.F. Mullenders

I. Objectives of the project:

This project is aimed at gaining information (1) on the nature of radiation induced DNA lesions and their repair and on the manifestation of genetical effects in mammalian cells, both under in vitro and in vivo conditions; (2) on the effect of radiation on germ cells of rodents and primates, as measured by induced chromosomal translocations, with the idea of using such data to estimate genetic risks due to radiation in man.

II. Objectives for the reporting period:

1. Inducible repair in human lymphocytes.
2. Study on chromosomal aberrations in germ cells of primates and rodents.
3. HPRT$^-$ mutant frequencies in patients that received a low dose of gamma irradiation from technetium-99m for a diagnostic test of heart function.
4. Kinetics of Repair of chromosomal damage following X-irradiation of human lymphocytes at Go stage.

III. Progress achieved:

Inducible repair in human lymphocytes

Methodology. The methodology used in these studies is the same as that described in the 1987 report. Briefly, in experiments in which radioisotopes were used to administer the "conditioning dose", 6 h after PHA stimulation of lymphocytes, ^{3}H-TdR (0.01 uCi/ml), tritiated water (5 uCi/ml), ^{14}C-TdR (0.01 uCi/ml) or ^{32}P (0.1uCi/ml) was added to the culture medium. The cultures were X-irradiated with 50 rad ("challenge dose) 50 h thereafter. Colcemid was added 1 h later and the cells were fixed at 53 h. In experiments on adaptive response with a low dose of X-rays, the cultures were exposed to 5 rad 32 h after stimulation, to 150 rad at 48 h and fixed 6 h later. 9 donors were used to obtain blood samples and the number of cells scored per group was at least 300.

Results.

Most of the data obtained using radioisotopes were presented in the 1987 EURATOM report. The additional results pertain to (i) one experiment with tritiated water (ii) one experiment with ^{32}P and (iii) one experiment with (2 donors) X-rays. Those from the X-ray study are given below:

Donor	Group	No. cells	chromatid breaks	Exchanges	Dic+rings
1	Control	400	12 (3.0%)	0	0
	5 rad	400	30 (7.5%)	2	0
	150 rad	394	105 (26.6%)	9	0
	5+150 rad	400	98 (24.5%)	8	1
2	Control	400	12 (3.0%)	2	0
	5 rad	500	31 (6.2%)	0	3
	150 rad	336	82 (24.4%)	8	1
	5+150 rad	400	83 (20.8%)	3	8

Discussion.

The results obtained with radioisotopes as well as with X-rays confirm and extend those presented earlier. They show that in the groups that received the "conditioning dose" and the "challenge dose", the yields of chromatid breaks are less than the sum of the yields expected on the basis of additivity of the effects of the individual treatments i.e., human lymphocytes can become "adapted" by prior exposure to low level irradiation so that they become less sensitive to the chromosome-breaking effects of X-rays delivered subsequently. The magnitude of reduction in frequencies in the "adapted" cells, however, varied between blood samples of different donors suggesting inter-individual variability in adaptive response. Bosi and Olivieri (Mut. Res, 1989 in press) have also demonstrated inter-individual variability in adaptive response between lymphocytes of different donors and those from the same donors sampled at different times.

Study on chromosomal aberrations in germ cells of primates and rodents

Methodology

To establish the position within the ovarian cycle, twice a week blood samples were taken from a female marmoset followed by determination of the progesterone level. Using different combinations of PMS (pregnant mare serum) and HCG (human chorionic gonadotrophin) it was tried to induce superovulation and to recover the maturated oocytes.

Male mice received local testis irradiation with 250 kV X-rays and meiotic preparations were made at appropriate sampling times after in vivo pulse labeling with H^{3}-thymidine.

Results:

Marmoset

Progesterone levels levels clearly indicated a 28 day ovarian cycle in the marmoset. The applied superovulation protocols derived from human and squirrel monkey data, were both negative for the marmoset, thus no oocyte recovery could be done.

Mouse

Preliminar data at the 6 Gy level showed that the labeling pattern of cells carrying radiation induced reciprocal translocations was different from that of normal cells.

Discussion

The recorded differences in labelling patterns indicated that translocation carrying cells were delayed in their progression through the meiotic stages thus leading to an overestimate of this type of genetic damage if spermatocyte analysis is used. At this moment the data do not allow quantification of the overestimation.

HPRT$^-$ mutant frequencies in patients that received a low dose of gamma irradiation from technetium-99m for a diagnostic test of heart function.

Recently, Seifert et al. 1987 (Mutation Res.,191, 57-63) presented data suggesting that exposure of nuclear medicine patients to ionizing radiation from technetium-99m results in an increase of the HPRT$^-$ mutant frequency in peripheral T-lymphocytes. Because of these quite alarming results, and the possible health risk to the patients from the treatment with technetium 99-m we decided to repeat these experiments.

Methodology

Patients were injected with sodium pertechnetate (750 MBq) and immediately thereafter multiple-gated scintigraphy was performed. Due to this examination an effective dose-equivalent of 2 mSv was estimated. Treatments were given in the Department of Radiology, Division of Nuclear Medicine, University Hospital Leiden under the guidance of Prof.Dr.E.K.J. Pauwels and Ing.J.A.J. Camps. 40 ml of heparinized blood was sampled immediately before technetium treatment and at 8-120 days thereafter (see Table 1).

Table 1.

Patient	Exposure (days)	-TG			+TG			Mutant frequency x 10^{-6} (95% Cl)
		Pos. wells	Total wells	Cloning efficiency	Pos. wells	Total wells	Cloning efficiency x 10^{-6}	
1	before	236	384	0.48	51	960	2.73	5.7 (4.2, 7.8)
	after (8)	115	384	0.18	20	960	1.05	5.9 (3.7, 9.5)
2	before	29	384	0.04	17	672	1.28	32.8 (18.0,59.8)
	after (15)	43	384	0.06	10	480	1.05	17.8 (9.0, 35.5)
3	before	26	384	0.04	15	960	0.79	22.5 (11.9,42.5)
	after (14)	33	384	0.05	16	953	0.85	18.8 (10.4,34.2)
4	before	210	384	0.40	45	960	2.40	6.1 (4.4, 8.4)
	after (14)	262	380	0.59	62	960	3.34	5.7 (4.3, 7.6)
5	before	148	384	0.24	13	336	1.97	8.1 (7.3, 9.1)
	after (120)	320	384	0.90	64	960	3.45	3.9 (2.9, 5.1)

We isolated mononuclear cells from the blood on Percoll and primed them for 40h (10^6/ml) in RPMI medium supplemented with serum, Hl-1, an Il-2 source (LAK supernatant), glutamine, PHA (mitogen) and antibiotics.
Primed cells were then seeded in two different ways: A) 4 cloning efficiency plates were used with 2 target cells/well and 20.000 irradiated TK6 feedercells and B) usually 10 plates were used for selection of mutants. In such plates each well contained 20.000 target cells and 10.000 irradiated feeder cells. The medium was the same as in the cloning efficiency plates except that 6-thioguanine was added.
$HPRT^-$ and $HPRT^+$ clones were screened visually after a culture period of 11 days.

Results and discussion

Results are presented in Table 1. In none of the patients investigated we found that the mutant frequency after treatment was higher than before treatment. Thus our results are clearly at variance with those of Seifert et al. 1987 (Mutation Res.,191, 57-63). In patient No.5 the mutantfrequency after treatment was even significantly lower than before treatment (no overlap of 95% confidence limits). In patients 2 and 3 the cloning efficiency was rather low. The most likely explanation for this is the old age of the donors (respectively 64 and 67 years old). In a control group of 20 non-smokers and 11 smokers the mean cloning efficiency was 37% (range 11.7-64.0). At present we are expanding the data base, but on the basis of the data obtained sofar we are confident that there is no evidence for adverse genetic effects of the extremely small dose of radiation given to patients for a diagnostic test of heart function.

Kinetics of Repair of chromosomal damage following X-irradiation of human lymphocytes at Go stage.

The frequency of chromosomal aberrations increases with dose and we have earlier produced biochemical and cytological evidence indicating that fast repairing DNA strand breaks lead to these aberrations. These studies have been extended using the Premature chromosome condensation technique (PCC).

Methodology

Isolated human lymphocytes were irradiated with 2 Gy and fused either immediately or after 30,60,90 and 120 min. with mitotic CHO cells to induce premature chromosome condensation. Chromosomal preparations were made of fused cells and they were stained by C banding technique enabling scoring of dicentrics and rings. Fractionation experiments were carried out with doses of 2 Gy and 3 Gy (split in two halves) with time intervals of 30 to 180 min. Part of the lymphocytes was processed for PCCs and part of them was cultured for 48h. and processed for analysis of metaphases.

Results and Discussion

Following 2 Gy, the observed frequencies of dicentrics and acentric fragments at different times of fusion are presented in Figs. 1 and 2. The frequency of dicentrics at 0 time was 28/100 cells and this remained more or less constant till 120.min. whereas the frequency of acentrics was 60/100 cells, which reduced gradually 22/100 cells by 120 min. These results indicate that most of the lesions which give rise to dicentrics are repaired very fast and residual breaks are repaired slowly. These results support our earlier conclusion on the involvement of short lived lesions in the formation of exchange aberrations (Natarajan et al, Mutation Res.160,231-236,1986). The frequency of dicentrics observed is very close to that observed at metaphase cells following 2 Gy irradiation. In fractionation experiments with 2 Gy, no difference in the total yield of dicentrics was observed with different intervals. However, with 3 Gy, there was a reduction in the frequency of dicentrics when doses were split at intervals of 120 and 180 min. Both PCC and metaphase data were

very similar. (Fig.3 & 4).
The contribution of dicentrics formed slowly is relatively low at 3 Gy.
All these data indicate that the contribution of lesions repairing slowly (such as base damages) towards the formation of exchange aberrations is minimal.

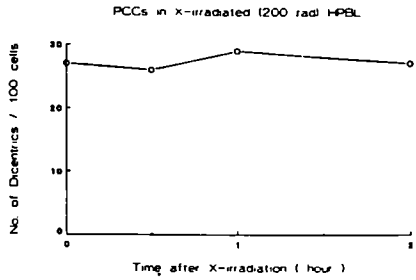


Fig. 1

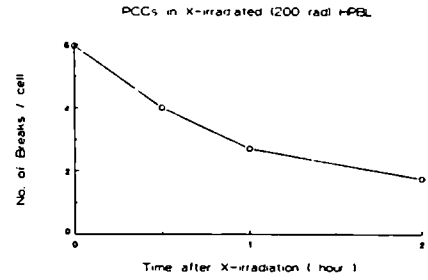


Fig. 2

Fig. 3 (PCC experiment):

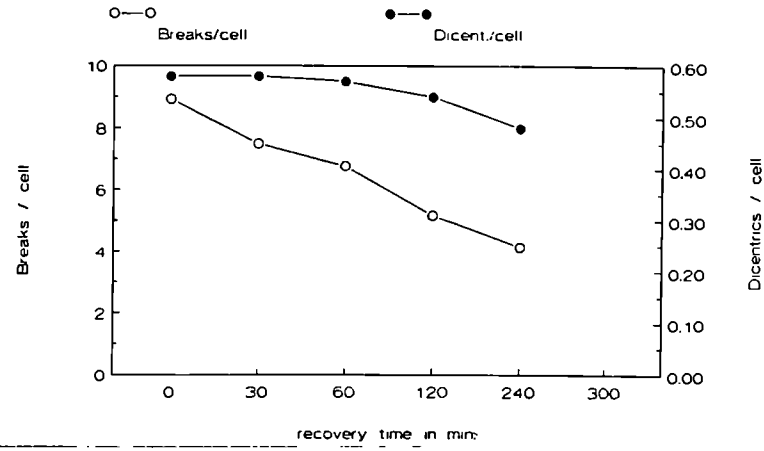
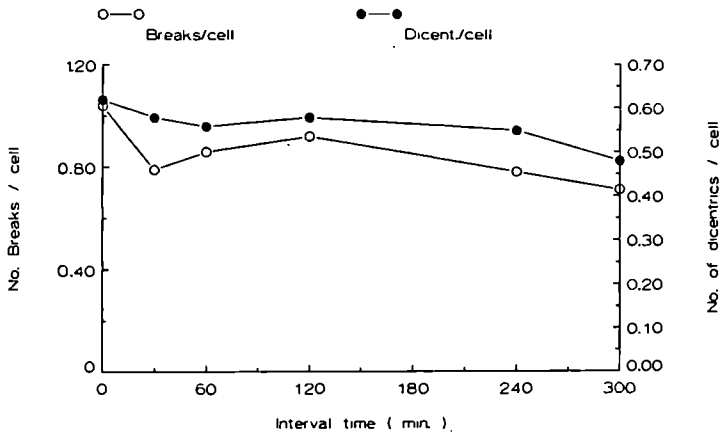


Fig. 4 (Metaphase experiment):



IV. Objectives for the next reporting period:

The objectives of this group of projects are to understand the relationship between radiation induced primary DNA lesions, their repair and genetic effects detected in germ cells and somatic cells of mammals (including primates and man). Therefore, further experiments will be designed on the basis of the results obtained in the last year and a outlined in our contract programme.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. I.F. Bernini, Dept. of Human Genetics, University of Leiden.
Prof. J.S. Ploem, Dept. of Cytochemistry and Histochemistry, University of Leiden.
Prof. F. Palitti, University of Rome, Italy.
Prof. G. Obe, Free University, Berlin, Federal Republic of Germany.
Dr. J. Cole, MRC Cell Mutation Unit, Brighton (U.K.)

VI. Publications:

Buul, P.P.W. and S.C. van Buul-Offers (1988) Effects of hormone treatment on chromosomal radiosensitivity of somatic and germ cells of Snell's dwarf mice. Mutation Res. 198, 263-268.

Buul, P.P.W. van (1988) Induction of chromosomal aberrations by ionizing radiation in mammalian germ cells. Proc. 29th Dutch Federation Meeting 62 (Abstract).

Buul, P.P.W. van (1988) On the relationship between X-ray-induced reciprocal translocations and cell killing of mouse stem cell spermatogonia. Int. J. Rad. Biol. 54, 844 (Abstract).

Sankaranarayanan, K. and P.P.W. van Buul (1988) Radionuclides and genetic risks. Int. J. Rad. Biol. 54, 842 (Abstract).

Natarajan, A.T. R.C. Vyas, F. Darroudi and L.H.F. Mullenders. The relation between DNA damage and chromosome aberrations - in Proc.XVth Berzelius Symposium, Umea, 1988 (in press).

Pohl-Ruling, J. O.A.Haas, G. Obe, A. Brogger, U. Roscher, F. Daschil, C. Atzmuller and A.T. Natarajan. The Charnobyl fallout in Salzburg/Austria and its effect on blood cell chromosomes. Proc. Int.Symp. on DNA repair, chromosome alterations and chromatin structure under environmental pollutions. U.S.S.R Academy of Sciences, Moscow. 1988 . Acta Biologica Hungarica (in press)

Karsdon,J., van Rijn, J, Berger, H. and Natarajan, A.T. Increased frequency of spontaneous and x-ray induced chromosomal aberrations in lymphocytes from neonates and the influence of caffeine- an in vitro study. Mutation Res. (in press).

Ramalho, A. I. Sunjevaric and A. T. Natarajan. Use of the frequencies of

micronuclei as quantitative indicators of X-ray induced chromosomal aberrations in human peripheral blood lymphocytes; Comparison of two methods. Mutation Res. 207 (1988) 141-146.

Ramalho, A., A.C. Nascimento and A.T.Natarajan. Dose assessments by cytogenetic analysis in the Goiania (Brazil) radiation accident. Radiat. Prot. Dosimetry. (in press)

Sankaranarayanan, K., A. v. Duyn, M. L. Loos and A. T. Natarajan. Adaptive response of human lymphocytes to low level radiation from radioisotopes or X-rays. Mutation Res., 1989, in press.

Title of the project no.: 5

Studies on mutations and their repair in Drosophila

Head(s) of project: Dr. J.C.J. Eeken

Scientific staff:

Dr. W. Ferro,
Dr. A. Pastink
Drs. J.G.R. de Cock

I. Objectives of the project:

(1) To investigate the mechanism by which heritable genetic damage such as mutations and chromosome aberrations arise from initial radiation-induced DNA lesions. (2) To study biochemically the production and processing of induced lesions in DNA repair-deficient mutants. Using the same repair-deficient strains, genetic and molecular techniques will be employed to determine the nature of recovered genetic endpoints. This approach is aimed at gaining a unified picture, integrating findings on DNA repair-defects with the responses observed in genetic experiments. (3) To study the influence of mobile elements and transposition events on specific radiation induced damage.

II. Objectives for the reporting period:

1. The repair of UV induced damage in individual genes in repair-proficient and repair-deficient cell lines (mei-9 and mus-201).
2. The genetic effect in somatic tissues of repair deficient mutations (mei-9, mei-41 and mus-101).
3. Isolation of white en vermilion mutations in a mei-41 background.
4. The molecular analysis of white mutations recovered in a mus-201 background.
5. The molecular analysis of chemically induced vermilion mutations.
6. Induction of bleomycine sensitive mutants.
7. Induction of P-insertion mutations at repair genes.
8. The effect of multi-locus deficiencies in heterozygous condition.

III. Progress achieved:

1. The repair of UV induced damage in individual genes in repair-proficient and repair-deficient cell lines (mei-9 and mus-201)

It has been shown in several investigations that DNA-repair is not randomly distributed over the genome (preferential repair of active genes). In addition to studies on the induction of mutations in germ line and somatic cells in repair-deficient Drosophila strains, we investigate the repair in individual genes (Gart, RpII, white and Notch) in cell lines derived from excision-repair deficient Drosophila's (mei-9, mus-201). GART is a 10 kb gene that is involved in purine synthesis. The gene product of RpII (9.4 kb) is the large subunit of RNA-polymerase II. From the literature, both genes are known to be actively transcribed in the Drosophila wild-type Kc cell line. White (6 kb) and Notch (34 kb) are supposed to be not transcribed in these cell lines. Here we report the removal of UV-induced thymidine-dimers in the GART and Notch genes.

Methodology

The removal of thymidine-dimers is measured by blot-hybridization. Cells are irradiated with 10 J/m² UV. After a period of repair-incubation (0, 8 and 24 hours) the DNA is isolated and purified. DNA is digested with restriction enzymes to obtain fragments on which an entire gene (Gart, Xho - 15 kb; RpII, $BstE_2$ - 9.4 kb; white, EcoRI - 15 kb) or part of a gene (Notch, Xho - 17 kb (intron) and - 15 kb (exon)) is situated. Part of the digested DNA is incubated with T4-endonuclease. This enzyme recognises thymidine-dimers and introduces specifically single strand breaks at these sites. T4-endonuclease treated and untreated DNA is fractionated on an alkaline agarose gel, blotted and hybridized with a probe of one of the four genes (Gart, RpII, white Notch). If no thymidine-dimers are present, a discrete band can be detected in the DNA independent whether this DNA is treated or not treated with T4-endonuclease. However, if thymidine-dimers are present in the selected fragment, than in the T4-endonuclease treated DNA, some DNA from the discrete band is cut and a smear of lower molecular weight DNA is detected. The ratio of DNA in the discrete bands of the T4-endonuclease treated and untreated DNA is a measure for the number of thymidine-dimers present in this DNA-fragment.

Results

It is found that both the active gene GART as well as the inactive gene Notch are not repaired in the mus-201 (excision-repair deficient) cell line. In the mei-9 (excision-repair deficient) cell line so far only the GART gene has been tested and like in mus-201 no repair was found.

Discussion

No repair of thymidine-dimers is found in the excision-repair deficient cell lines mus-201 and mei-9. These results are in agreement with the results obtained with other biochemical methods; i.e. these cell lines can not introduce single strand nicks at thymidine-dimers (in total genomic DNA) nor do they show Unscheduled DNA Synthesis (UDS) after UV-irradiation.

2. The genetic effect in somatic tissues of repair deficient mutations

The biochemical characterization of repair deficient mutants has been performed with somatic cells while the genetic effects of these mutants have been studied in cells of the germ line. To improve the correlation between these two levels it was decided to investigate the effects of repair-deficient mutants on the mutation induction in somatic cells.

Methodology

Larvae of repair deficient mutant strains were X-irradiated. The emerging adult flies were scored for (induced) clones of 1) white ommatidia in the

eyes of w/w^+ flies, or 2) multiple wing hair cells in wings of mwh/mwh^+ flies. The number and size of the clones was used to estimate mutation rates.

Results

The spontaneous mutation rate is 3-10 times higher in the cells of the wings as well as the eyes in mei-9 flies (excision repair deficient) than in repair proficient flies. After irradiation, the induced frequency of small clones (1-2 cells) in the wings (but not of the larger clones) is higher in mei-9 flies. In the eyes, the induction of small and large clones is higher in mei-9 flies after irradiation. In addition, the relative size of these induced clones is larger in mei-9 than in repair proficient flies.

Discussion

The increased mutation frequency, spontaneously as well as induced, in a mei-9 background is also found in germ line cells. This finding supports the notion that DNA repair in germ line cells and somatic cells functions identically.

The increased size of induced clones in the eyes in mei-9 relative to the control is the result of the increased sensitivity of mei-9 cells to killing by X-rays. The cells that survive irradiation have to divide more times to form the complete adult organs.

3. Isolation of white en vermilion mutations in a mei-41 background.

The data on the effect of mei-41 (post-replication repair deficient) on radiation induced SLRL mutations, translocations and Dominant Lethals in the germ line suggest that the spectrum of radiation induced mutations in mei-41 is different from that in a repair proficient background. In this project we will analyse the molecular nature of the lesions underlying the X-ray induced mutations recovered in a mei-41 background and compare this with the molecular nature of X-ray mutations that arise in a repair proficient situation. Here we report the isolation and genetic characterization of the mutations.

Methodology

Amherst-M56i wild type males are irradiated (15 Gy) and mated with females with the genetic constitution In(1)sc^{8}In(1)dl-49, y^{31d} w^a v^{of} mei-41. The F_1 females can be scored for induced X-linked mutations at the y (yellow body color), w (white eye color) and v (vermilion eye color) genes. The mutants are analysed and classified in intragenic mutations and gross chromosomal aberrations (multi-locus deficiencies, translocations, inversions) according to genetic and cytogenetic characteristics. Only mutations induced in mature sperm are collected.

Results

The number of irradiated X-chromosomes scored is 121,757 and the number of detected mutations at the y locus is 10, at the w locus 36 and at the v locus 42. Many of the F_1 females carrying one of these visible mutations are in addition sterile, indicating gross chromosomal aberrations. In case the detected mutation is heritable, the visible mutation can be associated with a recessive lethal mutation, indicating in most cases a multi-locus deficiency. Even if the visible mutation is not associated with a lethal mutation, it may still be associated with a genetic change rendering the males sterile. This characteristic is also indicative for a more severe damage underlying the mutation. In the following Table, X-ray induced mutations at the y, w and v locus are compared that were recovered in a repair proficient and a mei-41 background.

THE CLASSIFICATION OF X-RAY INDUCED MUTATIONS, WITH RESULTS OF FERTILITY AND TRANSMISSIBILITY TESTS

	y		w		wN	v	
	wb*	mos*	wb	mos	wb+mos	wb	mos
15 Gy, mei+, 108,291 F_1							
Exceptional females	17	3	18	11	15	41	0
Fertile	16		17		9	20	0
Mut. transmitted	16		16		6	20	0
F_2 mutant males Lethal	5		4		6	6	0
Viable	11		12		0	14	0
Sterile	2		1		0	1	0
15Gy, mei-41, 121,757 F_1							
Exceptional females	8	2	20	16	6	38	4
Fertile	7	2	14	1	4	12	4
Mut. transmitted	7	2	14	1	3	12	2
F_2 mutant males Lethal	2	1	5		3	6	0
Viable	5	1	9	1	0	6	2
Sterile	1		1		0	0	0

* wb = whole body mutations, mos = mosaic mutation

Discussion

The results of the genetic analysis indicate that the underlying lesion of X-ray induced mutations recovered in a mei-41 post-replication repair deficient background is more severe (more often large deletions) than when recovered in a repair proficient background. This result is opposite to that when X-ray induced mutations are recovered in an excision-repair deficient background (mus-201).

4. The molecular analysis of X-ray induced white mutations recovered in a mus-201 background.

Methodology

Amherst-M56i wild type males are irradiated (15 Gy) and mated with excision repair deficient (mus-201) females with the genetic constitution In(1)sc^{a}In(1)dl-49, y^{31d} w^{a} y^{of} f; mus-201. The F_1 females can be scored for (induced) X-linked mutations at the y (yellow body color), w (white eye color), v (vermilion eye color) and f (forked bristle) genes. Only mutations induced in mature sperm are collected. The mutants are analysed and classified according to genetic and cytogenetic characteristics in 1. intragenic mutations and 2. gross chromosomal aberrations (multi-locus deletions, translocations, inversions). DNA from whole body white mutants from the first class was analysed by molecular techniques.

Results

The number of irradiated X-chromosomes scored is 83,636 and the number of detected mutations (whole body and mosaic mutations) at the y locus is 7, at the w locus 63, at the v locus 15 and at the f locus 28. The frequency of whole body mutations recovered in an excision-repair deficient background appears to be the same as in a repair proficient background. The frequency of mosaic mutations however, is a factor 3-4 higher in the excision-repair deficient situation. The genetic analysis shows that of

the 17 transmitted whole body white mutations 3 are multi-locus deficiencies. Blot-analysis of the remaining 14 mutations showed a deletion of about 400 bp in one mutant and an inversion in another. In 8 mutants the mutation is a deletion smaller that 50-100 bp as was shown by S1 analysis. In one mutant only a base-pair change was observed. Three mutants with a normal restriction enzym pattern were not futher analysed.

Discussion

The increase in the frequency of mosaic mutations in a mus-201 background indicates that the excision-repair pathway normally is involved in the removal of at least part of the X-ray damage. In the absence of excision-repair part of this unrepaired or partially repaired damage is not a major impediment for DNA replication. Among the whole body mutations we observed an increase of the fraction of deletions not detectable by blot-analysis (in a mus-201 background about 50% and in a repair proficient background about 25%). These results may indicate that the excision-repair mechanism is also involved in the formation of large rearrangements.

5. The molecular analysis of chemically induced vermilion mutations.

Methodology

Vermilion mutants were induced by ENU or EMS treatment and cloned using the recombinational screening method. As a result of this method, the vermilion alleles are finally recovered on a plasmid from which single stranded DNA can be isolated. For each mutant the complete coding region was sequenced.

Results

In total we have analysed 23 ENU-induced mutants. Only base-pair changes were observed. Three mutants represent double mutations. Transition mutations were the most prominent class of sequence change: 62% were GC to AT and 17% were AT to GC substitutions. The spectrum also includes a significant fraction of transversion mutations (21%). In case of EMS a limited number of mutants was analysed. So far only GC to AT changes were seen.

Discussion

Both types of transition mutations can be explained by the miscoding properties of the O^6-ethylguanine and the O^4-ethylthymine adducts. Transversions can be explained by misrepair or miscoding properties of the modified bases.

6. Induction of bleomycine sensitive mutants.

A series of mutants of Drosophila melanogaster have been reported that are deficient in repair of UV induced DNA damage. Although the mutants alter in general the frequency of X-ray induced genetic damage, no repair defects could be demonstrated yet for X-ray induced DNA damage. Also the detected increases and decreases in the frequencies of X-ray induced genetic damage were relatively small. The selection of X-ray hypersensitive mutants was considered but was supposed to be complicated as X-rays are known to induce a wide range of DNA lesions. Instead bleomycin was chosen as the selection agent as it produces primarily DNA strand breaks which are also an important type of lesion induced by X-irradiation.

Methodology

Male flies were mutagenized with ENU and mated to attached-X females. The F_1 males were crossed individually to attached-X females and the F_2 cultures were treated with bleomycin. The absence or decreased frequency of males in the F_2 indicates a potential bleomycin sensitive mutant.

Results

So far about 9000 mutagenized X-chromosomes were screened. One mutant shows a clear hypersensitivity to bleomycin. 10 more mutants show a slight

hypersensitivity, while several putative mutants are in the process of being retested.

Discussion

The frequency of recovered bleomycin hypersensitive mutants is low compared to that of MMS hypersensitive mutants. As bleomycin induces far less different types of DNA damage than MMS, it is to be expected that mutations at fewer loci will confer bleomycin hypersensitivity. Presumably only a small number of loci are involved in the repair of DNA strand breaks.

7. Induction of P-insertion mutations at repair genes.

The mobile element P can be used to clone specific genes. Mutations due to the insertion of a P-element in a gene, will provide a 'tag' which can be used to isolate the DNA-sequences of the mutated gene. To clone DNA repair genes, mutations have to be made in these genes with the P-element.

Methodology

Dysgenic flies are generated in which the mobile element P is transposing at high frequency in germ line cells. Dysgenic males carrying a Pm balancer chromosome are crossed to C(1)DX females. The F_2 Pm males are mated individually to mus-201 (excision-repair deficient, MMS hypersensitive) females for 2 days after which the parental flies are transferred to new culture-vials. MMS is added to the first vial and scored for the presence or absence of Pm flies in the F_3. If a P-induced mus-201 is present on the Pm chromosome, only Pm^+ flies will survive the MMS treatment.

Results

So far we have tested about 12,000 second chromosomes and no P-induced mus-201 mutation has been detected.

8. The effect of multi-locus deficiencies in heterozygous condition.

Genetic risk for mutagenic agents is determined by (1) the number of mutations a particular agent induces per unit of dose and (2) the effect the induced mutations have in homo- and heterozygous condition. Those mutations that are expressed in heterozygous condition will, for obvious reasons, contribute much more to the relative genetic risk than those only apparent in homozygous condition. The first class of mutations with heterozygous effects are the Mendelian Dominants. The second class are recessive mutations influencing in minor or varying ways biological fitness. The importance of the second class of mutations is the relative abundance of this class over the Mendelian Dominants. In principle, we may expect recessive mutations to affect one gene (basepair changes, intragenic deletions and translocations and inversions) or several genes (multi-locus deletions). It has been pointed out repeatedly that multi-locus deletions could have larger heterozygous effects than single site mutations, implying that agents which are effective deletion inducers present a much higher genetic hazard than other mutagens. We tested several multi-locus deletions of about the same size, distributed randomly over the X-chromosome, as well as a number of deletions of various sizes in the same chromosomal region for their detrimental effect in heterozygotes.

Methodology

The basis of the measurement of a detrimental effect of a multi-locus deletion in a heterozygous condition in this investigation, is a competition between isogenic sister flies that differ only in that they carry the X-chromosome with the deletion or an X-chromosome derived from a wild-type laboratory strain, Canton S.

Results

The relative viability of deletion carrying females with different multi-

locus deletions are given in the following two tables. The size of the deletion is indicated as the number of salivary gland chromosome bands missing. First are listed the deletions that are localised at different regions of the chromosome.

Deletion	no. bands missing	rel. viability
svr	16-17	0.56
S39	16-18	0.51
TEM-75	16-18	0.78
N^{8}	17-19	0.63
JC70	22-28	0.67
HA32	16-18	0.52
v^{-64f}	16-18	0.92
N71	22-28	0.66
KA6	22-24	0.61
HF368	18-22	0.51

In the next table the deletions are listed that all are localised in the region of the X-chromosome around the gene white.

Deletion	no. bands missing	rel. viability
w^{67k30}	5-6	0.94
11-83	8	0.93
TEM-7	9	0.76
w^{N-59A}	8-12	0.96
B-3	13-14	0.86
TEM-75	16-18	0.78

Discussion

The results clearly show that the majority of these multi-locus deletions (in heterozygotes) have varying but pronounced effects on viability. The severity of this effect is not simply related to the size of the deletion. It is likely that there are genes, randomly distributed over the genome, that affect viability. If one of these genes is included in a particular deletion even though the deletion is small, the viability is reduced. The genetic risk for agents inducing multi-locus deletions is higher than agents inducing only intragenic lesions, because the chance of affecting one of the genes important for the viability of the individual is higher (at the same mutation frequency).

IV. Objectives for the next reporting period:

In the next reporting period we will focus on: (1) The repair of UV induced damage in individual active (RpII) and inactive (white) genes in repair-proficient and repair-deficient cell lines (mei-9 and mus-201). (2) The genetic effect of repair deficient mutations (mei-41 and mus-101) on somatic tissues. (3) The cytogenetic and molecular analysis of white en vermilion mutations in a mei-41 background. (4) The molecular analysis of chemically (EMS) induced vermilion mutations. (6) Induction and characterization of bleomycine sensitive mutants. (7) Induction of P-insertion mutations at repair genes. (8) The molecular nature of X-ray induced mutations in spermatogonial cells.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. E.W. Vogel, Dept Radiation Genetics and Chemical Mutagenesis, Univ. Leiden, Leiden, The Netherlands.
Dr. P.G.N. Kramers, R.I.V.M. Bilthoven, The Netherlands

VI. Publications:

EEKEN, J.C.J., A. PASTINK, M. NIVARD, E.W. VOGEL and F.H. SOBELS (1988) The nature of X-ray and chemically induced mutations in Drosophila in relation with DNA repair. In: Annual Review (M. Bignami and E. Dogliotti Eds.), Istituto di Sanita, Roma, (in press).

FERRO, W., and J.C.J. EEKEN (1988) Studies on mutagen sensitive strains of Drosophila melanogaster XI. Modification of X-ray induced dominant lethality and larval mortality by 7 repair deficient mutants. (in prep.)

PASTINK, A., C. VREEKEN, A.P. SCHALET and J.C.J. EEKEN (1988) DNA sequence analysis of X-ray induced deletions at the white locus of Drosophila melanogaster. Mutation Res., 207, 23-28.

PASTINK, A., C. VREEKEN, E.W. VOGEL and J.C.J. EEKEN (1988) Mutations induced at the white and vermilion loci in Drosophila melanogaster. Mutation Res., (in press).

PASTINK, A., C. VREEKEN and E.W. VOGEL (1988) The nature of ENU-induced mutations at the white locus of Drosophila melanogaster. Mutation Res., 199, 47-53.

SOBELS, F.H. and J.C.J. EEKEN (1988) Mutation induction by MR(P) and its modification by various conditions. In: Banbury Report 30: "Eukaryotic Transposable Elements as Mutagenic Agents" pp,195-207. Cold Spring Harbor Laboratories.

Title of the project no.: 6

The production of chromosome aberrations in human lymphocytes by low doses of X-rays

Head(s) of project: Prof. A.T. Natarajan

Scientific staff: Prof. K. Sankaranarayanan
Dr. A.D. Tates

I. Objectives of the project:

To irradiate blood in vitro to low doses of X-rays and to examine the lymphocytes in metaphase for radiation induced chromosome aberrations. The primary objective is to verify the existence of any low dose plateau in response over the range zero to a few tens of milligrays. Blood from 20 donors will be used because variations in sensitivity of donors may influence the low dose response. All cells containing exchange type aberrations will be photographed and karyotyped in order to determine whether certain chromosomes are specifically involved in such aberrations.

II. Objectives for the reporting period:

1. To complete scoring the material at 0 and 30 mGy
2. To decode and collate the results from the 6 collaborating laboratories
3. To analyse the data for:
   a) interlaboratory variation
   b) comparison of yields at 0 and 30 mGy
   c) possible donor variability
   d) multiply damaged cells
   e) karyotyping cells with exchange aberrations

III. Progress achieved:

The production of chromosome aberrations in human lymphocytes by low doses of X-rays

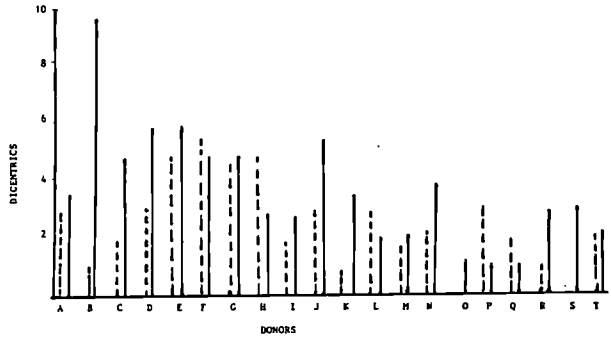
Methodology

Standard difference tests were applied to the data to determine the objectives a-c in section 2.

Results

Lab.	Dose (mGy)	No. Cells scored	Dicentrics	C. Rings	Excess Acentrics
	0	10500	14	1	63
1	30	10200	3	3	66
	0	10000	9	0	2
2	30	10000	11	1	8
	0	10015	10	0	28
3	30	9980	23	2	39
	0	10000	5	0	16
4	30	10000	11	1	17
	0	9797	8	0	16
5	30	9718	16	0	34
	0	10000	5	0	9
6	30	10000	10	0	26
Total	0	60312	51	1	134
	30	59898	74	7	190

The pooled donor results from the 6 laboratories.



The pooled laboratory results for dicentric aberrations in the 20 donors. For each donor 3,000 cells were scored for zero dose (broken lines) and 3,000 cells for 30 mGy (solid lines).

Discussion

As is usually experienced in collaborative projects some inter-laboratory differences in results are apparent. This is especially evident for the

acentric aberrations reported by laboratories 1 and 2. We have found no particular explanation for this divergence. The total numbers of dicentrics scored by each laboratory are reasonably consistent but laboratory 1 is exceptional in having found significantly more dicentrics in the controls than in the irradiated cells. This is mainly due to the very low yield at 30 mGy but the zero dose yield is a little high. The latter includes one cell containing 3 dicentrics from donor F (see later).

For acentric aberrations the inter-laboratory variations tend to cancel out and the total results are approximately consistent with a linear extrapolation of published dose response data. For dicentrics the background yield is consistent with previous reports of approximately 1 per 1000 cells. The yield in the irradiated cells however is low when compared with the data reported by this group of collaborating laboratories in a previous experiment (see section 6) and by extrapolating other published higher dose data assuming the linear quadratic model. Even if one excludes the odd result from laboratory 1 the yield is still low but tests show that the significance is marginal. These data therefore tend to support the idea of a threshold or plateau effect in the very low dose response.

A test on the distributions of the control and exposed sets of dicentric data shown in the Figure indicated no significant deviation from the Poisson distribution (zero dose $\sigma^2/y = 1.03 \pm 0.3$, 30 mGy $\sigma^2/y = 1.13 \pm 0.3$). This suggests that if inhomogeneity exists within this group of 20 subjects it could only be detected by many more cells being scored. There is a suggestion from the data in the Figure that subjects with a low control yield also exhibited a low irradiated yield and conversely a high control is associated with a high irradiated yield. However this trend is not quite significant but will be subject to further testing when data from two more doses become available.

Three multiply damaged (>1 dicentric) control cells were observed; one in each of donors F, H and J, and one irradiated cell in donor N. This is a similar finding to the previous experiment (section 6) and is again significantly greater than expected from Poisson statistics.

Karyotyping the cells containing exchange aberrations is still in progress but no result is yet available.

Because of the anomalies and variations described above it was resolved to score the other cells irradiated at 5 and 300 mGy that were prepared at the same time. The slides have been distributed to the collaborating laboratories and scoring is in progress.

IV. Objectives for the next reporting period:

To complete scoring the material irradiated at 5 and 300 mGy. To collate those results with the zero and 30 mGy data reported here. To analyse the full data in accordance with the objectives as described in sections 1 and 2 and to compare the results with those of a previous experiment now published (section 6).

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

National Radiological Protection Board, U.K. (Dr. D.C. Lloyd)
Free University of Berlin, Germany (Prof. G. Obe) (since transferred to University of Essen)
CEN/SCK Mol, Belgium (Dr. A. Leonard)
BNFL, Sellafield, U.K. (Dr. J. Tawn)
University of Rome, Italy (Dr. F. Palitti)

VI. Publications:

Lloyd, D.C., A.A. Edwards, A. Leonard, G.L. DeKnudt, A. Natarajan, G. Obe, F. Palitti, C. Tanzarella, E.J. Tawn (1988) Frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of X-rays. Int. J. Radiat. Biol., 53, 49-55.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Contract no.: BI6-E-226-NL

Head(s) of research team(s) [name(s) and address(es)]:

Prof. P.H.M. Lohman
Dept. Rad. Genetics & Chem. Mutag.
State University of Leiden
Wassenaarseweg 72
NL - 2333 AL Leiden

Telephone number: 071-276150/276151

Title of the research contract:

Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards.

List of projects:

1. Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards.

Title of the project no.: 1

Studies on spontaneously-arising genetic and partially genetic disorders in man within the framework of the evaluation of genetic radiation hazards.

Head(s) of project: Prof. Dr. K. Sankaranarayanan

Scientific staff: Prof. Dr. K. Sankaranarayanan

I. Objectives of the project:

1. To make a detailed analysis of the prevalence of spontaneously-arising diseases of complex aetiology, of Mendelian and chromosomal diseases (in that order) to examine the validity of the currently used prevalence estimates;
2. To make use of these data and those that bear on the severity of these diseases and arrive at estimates of detriment; and
3. To make an in-depth analysis of the mutation component of diseases of complex aetiology.

II. Objectives for the reporting period:

The principal objective of the work carried out thusfar has been (i) to make a detailed analysis of the prevalence of spontaneously-arising multifactorial diseases in Hungary, compare these estimates with those published in the literature and summarize their epidemiological, clinical and genetic features and (ii) to make use of these data and those on mortality and other apsects and arrive at estimates of detriment--handicap or harm at the individual and population levels.

III. Progress achieved:

Methodology. As mentioned in the 1987 EURATOM report, the 25 diseases selected for consideration represent a sub-set of those given in the WHO Manual on the International Classification of Diseases and Causes of Death. Based on clinical severity, these diseases have been divided into three groups, namely, group I, clinically very severe (schizophrenia, multiple sclerosis, epilepsy, acute myocardial infarction and systemic lupus erythematosus), group II, moderately severe and/or episodal or seasonal (Graves' disease, diabetes mellitus, gout, affective psychoses, glaucoma, essential hypertension, asthma, peptic ulcers, idiopathic proctocolitis, coeliac disease, calculus of the kidney, psoriasis, rheumatoid arthritis and ankylosing spondylitis) and, group III, less severe than those in groups I and II (varicose veins, allergic rhinitis, atopic dermatitis, Scheurmann disease and adolescent idiopathic scoliosis).

Baseline figures on population sizes, mortality and its causes were extracted from the Hungarian Demographic Year Books while data on age-standardized prevalences, mean ages at onset of these diseases and heritability estimates were based on epidemiologic studies carried out in Hungary. Data published in the literature were used to make comparisons with the Hungarian data.

The indicators used to assess detriment were: years of lost life potentially impaired life and actually impaired life. While the first two couldl be estimated from the epidemiologic data, for the third, use was made of data on premature retirement provided by the Office of Hungarian Medical Specialists.

Results and discussion.

A. Prevalence and mortality estimates. The results of our analysis of prevalence and mortality data were reported in the 1987 report. Briefly,(i) the total prevalence estimate for these diseases in Hungary is about 6500/10^4 individuals in the population (group I, 512/10^4; group II, 3218/10^4 and group III, 2811/10^4); (ii) this estimate is in agreement with published data from other countries, although there are differences with respect to some individual disease entities; (iii) the average annual mortality rates are 13.5/10^4 individuals for group I diseases, 6.68/10^4 for group II diseases and 0.03/10^4 for group III diseases and (iv) with the exception of epilepsy, in general, the amount of mortality is substantial between ages 20-69 and group III diseases are seldom causes of death.

B. Years of Life Lost (YLL). The mean number of YLL is substantial only for five of the diseases included in our list (epilepsy, 30 y; affective psychoses, multiple sclerosis and systemic lupus erythematosus, 18-20 y; and schizophrenic psychoses, 13 y. At the population level, the total YLL is about 2700/10^4 individuals with acute and subacute myocardial infarction accounting for about one-half of this total.

C. Years of potentially and actually impaired life (PIL and AIL). The mean PIL was calculated by subtracting the mean age at onset from the mean age at death. This covers a wide range (about 20-40 y, 12-70 y and 40-60 y for groups I, II and III, respectively), the overall mean being about 24 y. However, the nature and degree of impairment and the impact on the life quality of those afflicted differ for the different diseases. The mean AIL was estimated as the difference between the mean age at death and the mean age at premature retirement. This encompasses, again, a wide range from 16 to 45 y, but the overall averages for the diseases included in each of the three groups are roughly the same being about 20 y.

At the population level, the diseases considered herein cause about 96000 y of PIL and about 5800 y of AIL per 10^4 individuals in the population.

D. Comparison of estimates of detriment for multifactorial diseases with those for Mendelian diseases. For this purpose we used estimates of detriment arrived at by Carter (1982;Progress in Mutation Res 3, 1-8) for Mendelian and chromosomal diseases and by Czeizel and Sankaranarayanan (1984, Mutation Res 128, 73-103) for congenital anomalies. Our analysis shows that relative to Mendelian diseases as a whole, multifactorial diseases (congenital anomalies + the other multifactorials considered in this paper) are associated with much greater detriment (life loss: 1.4x; potentially impaired life years: 30x; and actually impaired life years: 3.9x).

IV. Objectives for the next reporting period:

In the next reporting period, we will focus attention on mental retardation in man (prevalence, aetiology and detriment analysis with respect to the Hungarian population and international comparisons).

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. A. Czeizel, Director, Department of Human Genetics and WHO Collaborative Centre for Genetic Diseases, National Institute of Hygiene, Gyali UT 2-6, H-1097, Budapest, Hungary

VI. Publications:

Sankaranarayanan, K (1988). Mobile genetic elements, spontaneous mutations and the assessment of genetic radiation hazards in man. In Banbury Report 30, Eucaryotic transposable elements as mutagenic agents (M. E. Lambert et al, Eds), 319-336.

Sankaranarayanan, K (1988). Invited review: Prevalence of genetic and partially genetic diseases in man and the estimation of genetic risks of exposure to ionizing radiation. Am. J. Hum. Genet 42, 651-652.

Czeizel, A.,K. Sankaranarayanan, A. Losonci, T. Rudas and M. Keresztes (1988). The load of genetic and partially genetic diseases in man. II. Some selected common multifactorial diseases: estimates of population prevalence and of detriment in terms of years of lost and impaired life. Mutation Res 196, 259-292.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-190-UK

United Kingdom Atomic Energy
Authority, UKAEA
11 Charles II Street
GB - London SW1Y 4QP

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Morgan
Environ. & Medical Sciences Div.
Harwell Laboratory
Didcot
GB - Oxon OX11 ORA

Telephone number: 0235-24141

Title of the research contract:

Cellular radiobiology.

List of projects:

1. Mutation and chromosome aberration in V79 cells by neutrons.
2. Cell transformation in C3H10T1/2 mouse cells by neutron beams.

Title of the project no.:

The effect of dose-rate on mutation frequency induced by 3.2 MeV neutrons at the HGPRT locus in V79 Chinese hamster fibroblasts.

Head(s) of project:

Dr A Morgan

Scientific staff:

Dr G R Morgan
Dr P D Holt
N Goberdahn

I. Objectives of the project:

To establish whether the effects of low doses of radiation on mammalian cells are independent of dose-rate.

II. Objectives for the reporting period:

To establish whether lowering the dose-rate enhances the mutation frequency induced in mamalllan cells by 3.2 MeV neutrons.

III. Progress achieved:

1 Methodology

V79 Chinese hamster cells were propagated and maintained in Eagle's minimum essential medium supplemented with 10% foetal calf serum, 2 mM glutamine, 50 $\mu g\ ml^{-1}$ streptomycin and 50 I.u. ml^{-1} penicillin. Plateau-phase cultures were prepared by seeding 25 cm^2 flasks containing 10 ml medium with approx. $5x10^5$ cells at least 84 hours before irradiation. Log-phase cultures were obtained in a similar way but the vessels were seeded with 1 to $2x10^5$ cells, 24-36 hours prior to irradiation.

A 5 MeV Van de Graaff accelerator provided a 3 MeV deuteron beam incident on a thick beryllium target. The cultures, positioned parallel to the horizontal axis of the target assembly, at 90^o to the incident deuteron beam, were irradiated with a neutron beam of mean energy 3.2 MeV when weighted by Kerma. High dose-rate exposures were given at 6-10 cGy min^{-1} and low dose-rate at 0.2-0.6 cGy min^{-1}. The cultures were maintained at 37^oC during the exposure. Controls and high dose-rate irradiations were sham-irradiated and the high and low dose-rate exposures were always paired.

The cells were recovered by trypsinisation within 5 minutes of the end of the irradiation. Measurements of the mutation frequency were conducted according to the methods of Thacker et al. (Thacker et al., 1977; Thacker and Stretch, 1983). Each of twenty 90 mm tissue culture dishes containing 10 ml medium supplemented with 6-thioguanine were seeded with 10^5 cells. At the same time sufficient cells to yield 200 survivors were seeded into each of five 90 mm dishes containing 10 ml medium to determine the surviving fraction. The survival dishes were incubated 7 days and the mutation dishes 10 days, before being stained with methylene blue. Colonies were counted by eye. On the mutation dishes, only colonies >2 mm diameter were scored. In some experiments the identity of the colonies was checked autoradiographically (Thacker et al., 1982) to ensure the size discrimination gave correct results.

2 Results

To ensure that the interval before mutants appeared in the population did not depend on dose-rate, the mutation frequency was monitored at various times after irradiation. No effect of dose-rate on expression time was observed for either plateau- or log-phase cultures. To obtain the yield of induced mutation, measurements made at three or more successive expression times between 4 and 10 days were averaged for each dose.

Plateau phase cultures Fig. 1 shows that the yield of mutants in plateau-phase cultures was proportional to dose and independent of dose-rate between 40 and 125 cGy.

Log-phase The reponse of log-phase cultures (Fig. 2) is generally similar to those in plateau-phase except at the lowest dose of 48 cGy. Here a low dose-rate consistently elicited a mutation frequency higher than the equivalent high dose-rate irradiation.

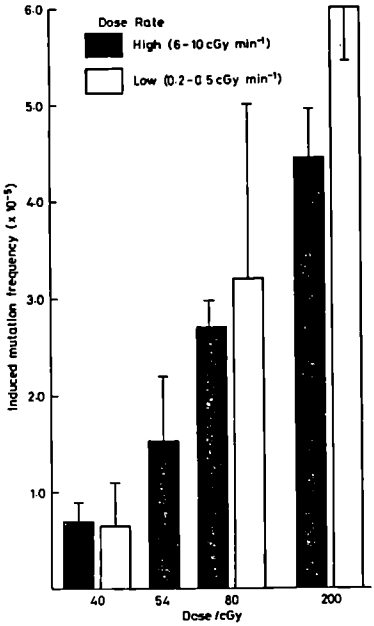


FIG. 1. MUTATION AS A FUNCTION OF DOSE-RATE OF 3.2 MeV NEUTRONS IN PLATEAU-PHASE CHINESE HAMSTER CELLS.

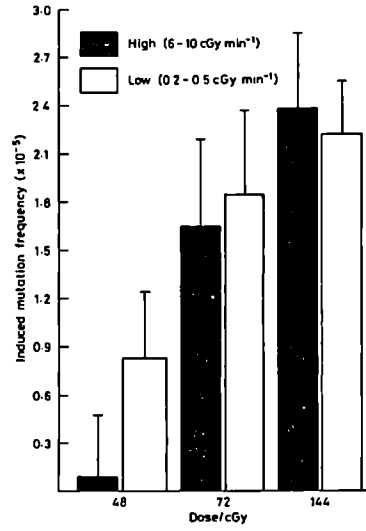


FIG. 2. MUTATION AS A FUNCTION OF DOSE-RATE OF 3.2 MeV NEUTRONS IN LOG-PHASE V79 CHINESE HAMSTER CELLS

3 Discussion

These results suggest mutagenesis at the HGPRT locus can show an inverse dose-rate effect under appropriate circumstances. The exact mechanism of the effect is still not clear but seems to require cell division. The range of doses over which the effect operates and its scale differs to that for transformation of C3H 10T½ cells (Hill et al., 1984) but whether this is a consequence of the cell line, the type of end-point or the radiation source is uncertain. Nevertheless, evidence is mounting that an inverse dose-rate effect might be a general phenomenon of high LET radiation. However, the effect may only be expressed under circumstances particular to each radiation quality.

References

Hill, C.K., Han, A. and Elkind, M.M. Int. J. Rad. Biol. 96, 11-15, 1984.

Thacker, J. and Stretch, A. Rad. Res. 96, 380-392, 1983.

Thacker, J., Stretch, A. and Brown., R. Mut. Res. 103, 371-378, 1982.

Thacker, J., Stretch, A. and Stephens, M.A., Mut. Res. 42, 313-326, 1977.

IV. Objectives for the next reporting period:

The work will be extended to α-particles. The mutation frequency will be analysed at doses ≤ 1 Gy and the effect of dose-rate measured. An effort will be made to try and improve an understanding of the phenomenon by investigating additional end-points.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None.

VI. Publications:

Conference proceedings

Morgan, G.R., Pepperall, D., Roberts, C.J. and Holt, P.D. The effect of dose-rate on the mutation frequency induced by high LET radiation in V79 Chinese hamster cells. In: Proc. 14th L.H. Gray Conf. Oxford 1988 (In press).

Title of the project no.:

Cell transformation in C3H 10T½ mouse cells by neutron beams

Head(s) of project:

Dr A Morgan

Scientific staff:

Dr P D Holt
Mr C J Roberts
Dr G R Morgan

I. Objectives of the project:

To establish whether a combination of low doses and low dose-rates can enhance transformation of mouse C3H 10T½ cells and whether this effect is a general feature of high LET radiation.

II. Objectives for the reporting period:

To further investigate the mechanism of transformation of C3H 10T½ cells and its susceptibility to environmental fctors. In particular, the effect of cell density on plating efficiency and its consequences for in vitro transformation measurements have been investigated. Also work has been carried out on a single cell assay to shed light on the variation of transformation frequency with cell density and to obtain an 'absolute' value of the transformation frequency.

III. Progress achieved:

1. Methodology

C3H 10T½ cells were maintained and propagated in Eagles Basal Medium supplemented with 10% FCS, 2 mM glutamine, 50 $\mu g\ ml^{-1}$ streptomycin and 50 i.u. ml^{-1} penicillin (complete medium). The stock cultures were incubated at 37°C in a 5% CO_2: air atmosphere.

Plating efficiency
For plating efficiency (PE) measurements, the cells were recovered from stock cultures by trypsinisation, counted and diluted appropriately in complete medium. Various numbers of cells were seeded onto either 90 mm dia. (63.6 cm^2) and 60 mm dia. (28.3 cm^2) culture dishes or 25 cm^2 tissue culture flasks, to yield cell densities ranging from 0.16 to 16 cells cm^{-2}. The cultures were incubated for 2 weeks before being stained with methylene blue and colonies containing more than 50 cells counted by eye.

Single cell assay for survival and transformation
To determine the growth of isolated cells, aliquots of cell suspension containing, on average, one cell were dispensed into the wells of ten microtitre dishes. The volume of complete medium in each well was made up to 0.2 ml and wells containing just one cell or a pair of cells in close proximity identified 2 days later. At the same time a series of ten 90 mm tissue culture dishes each received 960 cells. A further set of ten culture dishes each received sufficient cells (600) to yield 200 survivors as in the standard protocol for transformation. The microtitre dishes were incubated for 2 weeks then stained and wells containing colonies scored as survivors. Dishes were also stained and examined after 2 weeks and thereafter at weekly intervals for 6 weeks to record the progress of colony formation and the appearance of transformed foci.

2. Results

The PE of low numbers of 10T½ cells was influenced by the choice of culture vessel (see Fig. 1). 25 cm^2 flasks proved superior to either of the culture dishes, while the smaller sized dish offered a slight, but consistent, improvement over the larger. However, cell density profoundly influenced the PE. Increasing cell density depressed the PE on all three types of culture vessel. This effect was particularly striking on the 25 cm^2 flask where a four-fold increase in density from 4 to 16 cells cm^{-2} reduced the PE from >80% to <30%. The effect was present, but less marked, on the culture dishes. Taking the 90 mm dishes as an example, a 100-fold increase in cell density, from 0.16 to 16 cells cm^{-2}, was needed to reduce the PE by approximately 50%. In all three vessels the depression in PE appeared to be proportional to the logarithm of the cell density. On the culture dishes there was a suggestion of a greater effect at cell densities >10 cells cm^{-2}.

Effect of cell density on transformation frequency
Transformation frequencies were recorded under three different conditions:
a) with cells, physically separated from each other so that no cell-cell interactions could occur.

b) With an equal number of cells seeded onto 90 mm culture dishes so that the cell concentration was very low but interaction between cells was possible.
c) Under the standard conditions for a transformation experiment.

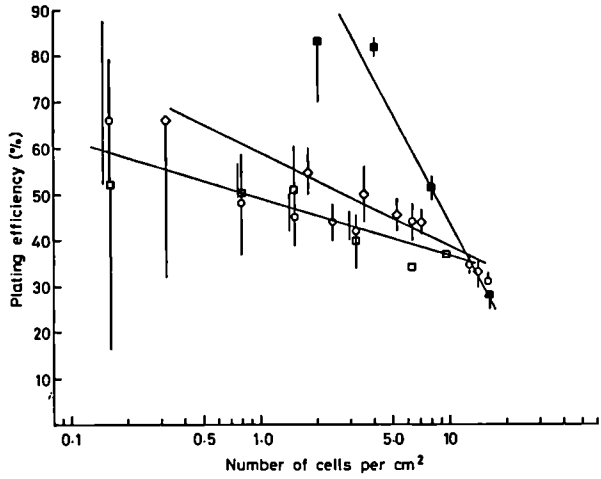


FIG. 1 Plating efficiency vs cell density for 25 cm^2 flasks (■), 60 mm dishes (◇), 90 mm dishes with cell line 1 (o) and 90 mm dishes with cell line 2 (□).

Colonies from single cells in the wells of a microtitre dish could be recognised as transformed by the same criteria as applied to a conventional 10T½ transformation assay. The transformation frequencies obtained under each set of culture conditions are recorded in Table 1. The spontaneous transformation frequencies were similar under conditions (a) and (b). In both cases the level of spontaneous transformation was significantly greater than on dishes prepared according to the standard protocol (c).

Table 1 Plating efficiency and transformation in different vessels

Culture vessel	Numbers of cells seeded	PE (%)	Transformation frequency (%)
Microtitre plate	96	27	35
90 mm dish	96	51	26
90 mm dish	600	21	7.3

3. Discussion

Survival of 10T½ cells cannot be measured directly in the population used to determine transformation rates. It is customary to prepare a parallel set of vessels containing fewer cells sampled from the same population used to seed the transformation dishes. However, this may be inappropriate as it is clear that the survival on the transformation dishes may differ from that on the survival plates and could be overestimated. The choice of culture vessel could compound the problem, as 25 cm^2 flasks appear to be much more susceptible to the effect than 90 mm dishes. Mixing the types of vessel for convenience could lead to the same result: survival on transformation dishes being overestimated in proportion to the ratio of the plating efficiencies of the corresponding cell concentrations leading to underestimation of the transformation frequency per surviving cell. The reason for this effect is not clear at present.

It has been known for some time that the transformation frequency depends on cell density. To try and overcome this problem, and validate the spontaneous transformation rate, a single cell assay was developed using microtitre plates. A 10T½ strain with a high level of spontaneous transformants was used for these experiments to improve their sensitivity. The PE of cells plated singly in the microtitre plates, or at low density on 90 mm dishes, was similar and both conditions yielded equal numbers of transformants suggesting the maximum yield of transformants could be observed at low cell densities. Physical separation of the cells conferred no advantage. However, both conditions gave significantly higher transformation frequencies than the standard protocol indicating that transformants were suppressed by that procedure. It remains to be determined whether a reduction in cell density due to cell killing by irradiation, would allow these pre-existing transformants to be expressed.

IV. Objectives for the next reporting period:

The transformation frequencies induced by high and low dose-rates of fast neutrons (mean energy 3.2 MeV) and α-particles will be measured and compared, using the standard technique.

The single-cell technique will be developed to determine whether radiation-induced cell killing increases the expression of spontaneous and induced transformants, and to provide correction factors to derive absolute transformation frequencies from data obtained using the standard technique. These correction factors will be applied to the neutron and α-particle data.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

The MRC Radiobiology Unit, Chilton (Dr D T Gooodhead) are collaborating on this project. They have supplied the ^{238}Pu α-particle source and have done all the dosimetry. They are collaborating in the interpretation of the results.

Liaison is also maintained with the Central Electricity Generating Board, Berkeley Nuclear Laboratories.

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-E-151-F

Institut Curie
Section de Biologie
Rue d'Ulm, 26
F - 75321 Paris Cédex 05

Head(s) of research team(s) [name(s) and address(es)]:

Dr. E. Moustacchi
Section de Biologie
Institut Curie
Rue d'Ulm, 26
F - 75321 Paris Cédex 05

Telephone number: 4329.03.76

Title of the research contract:

Comparison of the fate of X rays and DNA cross-linking agents induced lesions: Fanconi's anemia as a model of human repair defect. Genetic and biochemical analysis.

List of projects:

1. Complementation analysis by cell fusion in Fanconi's anemia. Search for complementation of defective repair functions by DNA transfection.
2. Inducibility of repair functions in FA fibroblasts.

Title of the project no.: 1

Head(s) of project: **Dr. Ethel MOUSTACCHI**

Scientific staff:

C. Diatloff-Zito	D. Fraser
D. Papadopoulo	B. Porfirio
S. Nocentini	J. Heddle
S. Rousset	F. Rosselli
D. Averbeck	E. Moustacchi

I. Objectives of this project:

Cells from Fanconi's anemia (FA) patients have an increased spontaneous frequency of chromosome aberrations and a hypersensitivity to DNA cross-linking agents. Because of this increased sensitivity, it is believed that FA is caused by a defect in a gene(s) concerned with the processing of DNA lesions. Taken together with cancer-proneness, this is the reason why FA is classified with diseases such as xeroderma pigmentosum and ataxia telangiectasia. It has been difficult to clearly define the basic biochemical abnormalities associated to these defects and on the other hand genetic studies have shown the existence of multiple complementation groups for these disorders. Certain mutant rodent cell lines also show an increased susceptibility to DNA-damaging agents and it has been possible to use DNA-mediated gene transfer to complement the cellular defect and to use molecular techniques to clone the sequences responsible for the correction of the cellular phenotype (Westerfeld et al. ; Rubin et al.). These results have encouraged us to attempt similar strategies with regard to the gene(s) defective in FA. In parallel, several cellular and genetic characteristics of FA and of FA-like mouse lymphoma mutants necessitated to be defined.

II. Objectives for the reporting period:

Further characterization of FA cell lines of genetically defined complementation groups and expansion of the culture collection in view of genetic definition. Parallel studies on a mouse mutant cells.

Strategies for transfer of mitomycin c resistant genes to human cells.

III. Progress achieved :

Progress toward the cloning of gene(s) involved in the response to DNA cross-linking agents.

a) Analysis of FA group B transfectants with an increased mitomycin C (MMC) resistance. The hypersensitivity to MMC of FA fibroblasts from the genetic complementation group B can be partially corrected by transfection of high molecular weight DNA from mouse lymphoma cells. The primary transfectants showed a restitution to about 50% of the normal MMC resistance, but no correction of the clastogenic effect of the drug. The acquired MMC resistance was stable over at least 4 months. Secondary transfectants were also generated and displayed a slightly lower MMC resistance than the primary ones. By Southern blot analysis, it is shown that primary transfectants contain mouse repetitive DNA sequences, the majority of which are lost following serial passages.

b) Analysis of a genomic library from FA group B transfectants. A genomic library in λ phage vector was generated from the primary transfectants which contain the lowest amount of mouse repetitive DNA sequences. The library (7.10^{-5} recombinants) was screened with an oligonucleotide specific of mouse repetitive DNA sequences. 56 phages hybrizing to the probe were picked. 36 over the 56 λ recombinant phages hybridized strongly to the probe, they were purified and amplified. The DNA of each λ recombinant was mapped for the localization of mouse and human repetitive DNA sequences. In parallel, their biological activity was tested by transfecting FA group B lymphoblastoid or fibroblastic cells. Three λ recombinants gave an increased MMC resistance of FA cells upon transfection. The magnitude of the MMC resistance recovery can vary from one experiment to the other depending probably upon the integration site. No λ recombinant appears to contain the entire gene, transfection experiments with mixture of phages are in progress.

c) DNA transfection into MCS^{-}_{1} mouse mutant cells by electroporation. Determination of selection conditions. Optimum conditions for electroporation of MCN 151 cells were determined using the electropulsing unit EMBL. 50% of the cells were electroporated with 30% mortality with 3 pulses at 4 or 5 kV and a capacity of 30 nF. Several rounds of transfection of the human cDNA library of Chen and Okoyama (Mol. Cell. Biol. 7, 2745, 1987) were applied to the MCN 151 mutant cells. The transfected cells were selected for resistance to the antibiotic G 418. One hundred such clones have been isolated with a frequency of $3\text{-}4.10^{-5}$. Each clone was then individually tested for potential correction of the sensitivity to MMC by growth in the presence of the drug. Since G 418 resistant clones grew at variable rates and that some were significantly slower than MCN 151 untransfected cells, false negative results could be obtained. Search for other selection conditions to isolate MMC resistant cells as well as experiments aiming at optimization of clonal selection conditions in 96 wells microtiter plates are in progress.

d) Preparation of a human cDNA library in an Epstein-Barr virus (EBV) shuttle vector. Recently transfection and expression of cDNA libraries in human cells has been achieved with EBV based subcloning vectors (Margolskee et al., 1988). The EBV subcloning vectors allow to directly recover a cDNA by selecting for its expression upon transfection of the whole library in various human cells and especially in lymphoblastoïd cells which can be transformed at high efficiency by such plasmids. The plasmids replicated as autonomous episomes in EBV transformed cells. A cDNA library in an EBV-shuttle vector is transfected in FA lymphoblastoïd cell line HSC 99 for the correction of MMC hypersensitivity. In preliminary experiments, determination of conditions for electroporation and expression of the vectors are in progress. Two EBV plasmids with different promotors (gift of M. James, laboratory of A. Sarasin, Villejuif, France) will be compared for their capacity to transform FA cells to hygromycin resistance, and for the expression of chloramphenicol acetyl

transferase enzyme (CAT assay). The library will be constructed in the most performant plasmid.

Expansion of the FA cell culture collection : analysis of genetic and phenotypic heterogeneity in FA. Skin biopsies derived from 22 FA patients have been developped into fibroblast cultures. They were submitted to the test of semi-conservative DNA synthesis measurements following treatment with a cross-linking agent (psoralen + UVA) (Moustacchi et al., 1987). According to this test, about 30% of the FA cell lines demonstrated the phenotype of the genetic complementation group A which is the most defective according to parameters related to processing of DNA cross-links. In view of a genetic linkage molecular analysis in FA families, when suitable probes will be available, material from FA relatives is collected.

Further analysis of the MCS mouse lymphoma mutants confirms the analogies with FA.

a) We show that after exposure to mitomycin C, the mouse lymphoma mutant MCS_1 previously shown to be hypersensitive to the toxic effect of DNA cross-linking agents (Hama-Inaba et al., 1988), demonstrates higher frequencies of chromatid-type aberrations and of aberrant cells in comparison to L5178Y cells. The dose-effect relationships as well as kinetics show strong analogies with the behaviour of FA cells in terms of the cytogenetic response.

b) By co-cultivation of normal human cells with MCS_1 mouse mutants, we show that there is a correction of this chromosomal instability of the mouse cells. The same is true when FA group B cells are co-cultivated with MCS_1 mouse mutant cells whereas such a correction is not observed by co-cultivating FA group A cells with MCS_1. In other words, normal and FA type B cells but not type A cells release in the growth medium a diffusible substance capable to abolish the cytogenetic anomalies of MCS_1 cells. It is tempting to equate the FA group A human defect with the MCS_1 mouse mutation.

c) Up to now, attempts of genetic analysis by somatic hybridization between mouse lymphoma and human fibroblasts or lymphoblasts have failed in our hands, due to the high chromosomal instability of the hybrids that we observed.

The basal and the mutagen-induced levels of two enzymes involved in DNA repair are not modified in Fanconi's anemia cells. The activities of DNA ligase and ADP-ribosyl transferase; two enzymes which have been claimed to be defective in FA have been investigated in normal and FA cells. Fibroblasts and lymphoblasts treated with cross-linking agents were compared to untreated cells (in collaboration with Dr. A. Sarasin group for the DNA ligase study and with Dr. U. Bertazzoni group for the ADP-ribosyl transferase study). The basal level of the two enzymatic activities as well as the response to treatments with DNA-damaging agents were similar in normal and FA cells. Consequently it is unlikely that the molecular defect in FA cells is due to a decreased activity of these two enzymes.

IV. Objectives for the next reporting period :

Characterization of the mouse DNA sequences which lead to partial correction of the FA phenotype in terms of the hypersensitivity to DNA cross-linking agents and growth characterization.

Analysis of the dissociation of functions (cytogenetic and cytotoxicity).

V. Other research group(s) collaborating actively on this project (name(s) and address(es)) :

- Prof. J. A. HEDDLE (Department of Biology, York University, Toronto, Canada).
- Dr. M. Z. ZDZIENICKA (Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Leiden, the Netherlands).
- Dr. U. BERTAZZONI (Istituto di Genetica Biochimica Evoluzionistica del CNR, Pavia, Italy).
- Dr. A. Sarasin (Institut de Recherche sur le Cancer, Villejuif, France).

VI. Publications :

- High levels of 4,5',8-trimethylpsoralen (TMP) photoinduced furan-side monoadducts can block cross-link removal in normal human cells.
  D. Papadopoulo, D. Averbeck and E. Moustacchi.
  Photochem. Photobiol., 47, 321-326 (1988).

- Sequence specificity in photoreaction of various psoralen derivatives with DNA : role in biological activity.
  V. Boyer, E. Moustacchi and E. Sage.
  Biochemistry, 27, 3011-3018 (1988).

- Sequence specificity in psoralen-DNA photobinding.
  E. Sage, V. Boyer and E. Moustacchi.
  Biochem. Pharmacol., 37, 1829-1830 (1988).

- Repair of 4,5',8-trimethylpsoralen plus light induced DNA damage in a normal and Fanconi's anemia cell lines.
  D. Averbeck, D. Papadopoulo and E. Moustacchi.
  Cancer Res., 48, 2015-2020 (1988).

- Survival and mutagenic responses of mitomycin C sensitive mouse lymphoma cell mutants to other DNA cross-linking agents.
  H. Hama-Inaba, K. Sato and E. Moustacchi.
  Mutation Res., 194, 121-129 (1988).

- Phenotypic and genetic heterogeneity in Fanconi's anemia, fate of cross-links, correction of the defect by DNA transfection.
  E. Moustacchi, D. Averbeck, C. Diatloff-Zito and D. Papadopoulo.
  In : "Fanconi's anemia : clinical and experimental aspects". Eds. : T. Schroeder-Kurth and G. Obe, Springer-Verlag, Berlin, pp. 196-210 (1988).

- Fanconi's anemia : Genetic and molecular studies.
  E. Moustacchi, D. Papadopoulo, D. Averbeck, C. Diatloff-Zito, S. Rousset and S. Nocentini.
  In : "Mechanisms and Consequences of DNA Damage Processing", Alan R. Liss Inc., pp. 371-380 (1988).

- Radiocancérogénèse et réparation de l'ADN.
  E. Moustacchi.
  In : "Cancérogénèse par les faibles doses de radiations ionisantes et normes de sécurité", Académie des Sciences, Paris, pp. 7-11 (1988).

Title of the project no.: 2

Head(s) of project:

Dr. Jacques COPPEY

Scientific staff:

Jacques COPPEY
Bernard LOPEZ
Maria SALA-TREPAT

I. Objectives of the project:

The high susceptibility of Fanconi's anemia (FA) cells to DNA cross-linking agents can result from alterations in the processing of interstrand cross-links. As such a processing involves a recombinational pathway, we have analyzed this pathway :

- in cultured cells by analyzing multiplicity reactivation and fidelity of reactivation with trimethylpsoralen photo-damaged Herpes virus
- in human nuclear extracts by studying the steps of homologous recombination and the requirements for recombinational repair of double-strand breaks.

II. Objectives for the reporting period:

1. Characterization of the protection process in the recombining DNA
2. Enzymatic and structural requirements for recombinational repair of double-strand breaks.

III. Progress achieved:

1. We have shown that extracts from human transformed cells (3 epithelial lines ; one lymphoblastoïd line) contain activities which lead to targeted replacement of an altered gene sequence in the replicative form (RF) of a derivative of M13 phage (M13 mp8) by the functional sequence purified from wild M13 mp8. The process takes place by single-strand exchange and can be reciprocal (Lopez et al., Nucleic Acids Res., 15, 5643-5655, 1987). Sequence analysis of 20 recombined clones over 400 nucleotides per clone, in a genetically silent sequence (hence with no selection bias), including the recombination initiation or termination sites, shows no base modification compared to the parental sequence despite nuclease/nickase activities present in the extract. A DNA protection against DNase I is transiently brought by protein(s) present in the nuclear extracts and is strickly limited to the homologous sequences potentially involved in recombination (Lopez and Coppey, in press). This observation may indicate that the recombining DNA's present a deoxyribonucleoprotein (dRNP) structure. We are now purifying the dRNP protein(s) with the aim at precisely stating their function : helicase, resolvase, transferase and/or DNA topoisomerase(s).

2. The repair of a defined double-strand break introduced by restriction in the M13 mp8 RF occurs preferentially via a recombinational pathway with an homologous duplex DNA. Exonuclease and polymerase activities are tightly coupled to this process. Its initiation requires structural constaints : 5' protruding or blunt ends (Lopez and Coppey, Nucleic Acids Res., 15, 6813-6826, 1987). Homology requirements bordering the cut for the initiation of recombinational repair have now been established : between 15 and 27 bp for a 8 bp heterology ; slightly longer (work in progress) for 160 or 950 bp heterologies.

The polarity of the associated exonuclease is presently examined by measuring the extent of recombinational repair following digestion of the free ends in the cutted substrate by defined exonucleases (from or T_4 bacteriophages). The extent of action of the associated exonuclease is estimated by measuring the incorporation of radioactive dCTP at the end of the repairing break.

IV. Objectives for the next reporting period:

The in vitro recombinational activity of human cells extracts from different pathological origins and especially from Fanconi's anemia cells will be examined. In this last case, genetically well defined complementation groups will be analyzed.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

- Multiplicity reactivation and mutagenesis of trimethylpsoralen damaged herpes virus in normal and Fanconi's anemia cells.
  J. Coppey, M. Sala-Trepat and B. Lopez.
  Mutagenesis, 4, 1988 (in press).

- Molecular analysis of homologous recombination catalysed by human cells extracts : fidelity and DNase protection.
  B. Lopez and J. Coppey.
  Biochem. Biophys. Res. Commun., 1988 (in press).

- Duplex-duplex homologous recombination catalysed by human nuclear extract. Involvement in double-strand break repair.
  B. Lopez and J. Coppey.
  Proceedings of a NATO Workshop : "DNA repair mechanisms and their biological implications in mammalian cells". Eds. : M. Lambert et al., in press, 1989..

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Consiglio Nazionale delle Ricerche
Piazzale Aldo Moro 7
I - 00185 Roma

Contract no.: BI6-E-158-I

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Nuzzo
Istituto di Genetica Biochimica
ed Evoluzionistica del CNR
Via Abbiategrasso 207
I - 27100 Pavia

Dr. U. Bertazzoni
Ist. di Genetica Biochimica
ed Evoluzionistica del CNR
Via Abbiategrasso 207
I - 27100 Pavia

Telephone number: 382.422.411

Title of the research contract:

Molecular and genetic analysis of DNA damage.

List of projects:

1. Proteins and structures of DNA replication and repair in animal cells, isolation of mammalian mutant cells altered in DNA metabolism and sensitivity to mutagens, and molecular analysis of modified DNA.

Title of the project no.:

Proteins and structure of DNA replication and repair in animal cells, isolation of mammalian cells altered in DNA metabolism and sensitivity to mutagens, and molecular analysis of modified DNA.

Head(s) of project:

Prof. U. BERTAZZONI, CEC Official
Dr. M. STEFANINI

Scientific staff:

Prof. F. NUZZO	Dr. A. CASATI
Dr. A.I. SCOVASSI	Dr. R. IZZO
Dr. C. MONDELLO	Dr. P. LAGOMARSINI
Dr. E. BOTTA	Dr. R. RIBONI

I. Objectives of the project:

Study of the structure and function of proteins involved in DNA repair in mammalian cells. Understanding the possible relationship between the mechanism of ADP-ribosylation of nuclear proteins and modification of chromatin structure after cell treatment with DNA damaging agents. Cloning of the gene for the enzyme ADP-ribosyl transferase and study of its structure and expression in normal and DNA repair deficient human cells.

Identification and analysis of DNA repair defects in cells from patients affected by hereditary diseases and in CHO clones hypersensitive to mutagens. Cloning of human repair genes complementing the defects present in the UV sensitive (UV^S) mutants.

II. Objectives for the reporting period:

Study of nuclear ADP-ribosyl transferase (ADPRT) in rat liver carcinogenesis (in coll. with F. Cesarone, Genova). Analysis of ADPRT activity and expression in synchronized HeLa cells (in coll. with H. Suzuki, Verona). Study of interaction between DNA and ADPRT (in coll. with S. Shall, Brighton).

Cellular and genetic characterization of UV-sensitive Chinese hamster mutants. Analysis of various cellular parameters used to detect genetic instability in patients affected by nevoid basal cell carcinoma syndrome (NBCCS). Genetic analysis of the defect conferring UV sensitivity in patients affected by trichothiodystrophy (TTD).

III. Progress achieved:

Methodology

Experimental models for in vivo carcinogenesis included the Teebor and Becker system of discontinuous 2-AAF treatment, the Solt and Farber model of mutagen treatment followed by partial hepatectomy and the Druckrey model based on diethylnitrosamine (DENA) treatment. HeLa cells were synchronized in S-phase by double block with aphidicolin. For the extraction of mRNA from tissues and cells, a guanidine-isothiocianate method was used. Analysis of mRNA of ADPRT was made on Northern blot using a cDNA probe. ADPRT was assayed on activity gel system.

The frequency of spontaneous chromosome breakage, the mutation frequency for 6-thioguanine (6TG) and the response to mitogens in lymphocytes exposed to mutagenic agents in Go phase were evaluated following standard procedures. Sensitivity to mutagens was studied by measuring unscheduled DNA synthesis (UDS), survival, and recovery of DNA and RNA synthesis. Genetic analysis was performed by measuring the UDS in the heterokaryons or the survival after UV light in the hybrids.

Results

The results obtained by using the Teebor and Becker model of rat hepatocarcinogenesis, showing a loss of ADPRT activity, were confirmed and other models were experimented, namely those of Solt and Farber and of Druckrey. In the first system a single injection of DENA resulted in a significant decrease of ADPRT activity and the subsequent administration of 2-AAF induced a further drop. Concerning the Druckrey model, no significant variation in enzyme band intensity was noted after 2,4,6 and 12 weeks of oral administration of DENA.

The functional role of ADPRT during the cell cycle was studied in synchronized HeLa cells. The intensity of the 116 kDa band of the enzyme on activity gel tended to remain constant during the whole S period and G2 and M phases. No significant variations were observed for the level of mRNA of ADPRT on the same samples.

The treatment of cellular extracts and purified preparations of ADPRT with DNase I caused a marked decrease in activity and the addition of exogenous DNA could not restore the enzyme activity found in untreated extracts.

Genetic analysis of the DNA repair defect in six UV^S CHO clones indicated that three mutants belong to group 1, one belongs to group 5 whereas the last two represent two new complementation groups. Attempts to isolate the genes that correct the defect in these two mutants were so far unsuccessful since the low mutagen hypersensitivity of the cells does not provide an efficient selection system for cells transfected with human DNA. To localize on human chromosome the gene able to complement the repair defect, hybrids between the mutants and human lymphocytes were obtained and their characterization for human chromosome content and UV sensitivity is in progress.

No evidence of genetic instability was found in lymphocytes from four NBCCS patients. All the parameters analyzed (the level of UV-induced DNA repair synthesis, the DNA replication rate after treatment with mutagens inducing different kinds of damage in the DNA molecule, the baseline mutation frequency and the spontaneous chromosome breakage) showed normal values; only the response to mitogens was delayed in comparison to that in normal donors.

DNA repair studies in fibroblasts from three English patients affected by trichothiodystrophy (TTD) indicated that one cell strain was repair proficient and able to complement the UDS defect in the repair-deficient TTD cells. The other two cell strains were repair deficient, the UDS level being reduced to 10% and 50% of normal. Complementation analysis demonstrated in both cell strains the presence of XP-D mutation.

Discussion

The enzyme ADP-ribosyl transferase in known to play a central role in the modulation of cellular response to DNA damage. The effect of DENA, a carcinogenic initiator, and of the subsequent administration of promoter 2-AAF on ADPRT activity was studied on liver of rats treated according to the model of Solt and Faber. ADPRT activity was strongly decreased after treatment with the promoter, thus confirming the results previously obtained using the Teebor and Becker model. On the contrary, when DENA alone was continuously given to rats according to Druckrey model, the activity of the enzyme remained constant, suggesting that the promoter is needed in order to produce an effect on ADPRT activity.

No significant variation in ADPRT activity was observed during the progression of the cell cycle in HeLa cells. These results were confirmed by measuring the level of mRNA for ADPRT, showing that the extent of enzyme expression tended to remain constant. The results obtained by studying the properties of ADPRT after DNase treatment indicate that the enzyme-bound DNA is essential in the regulation of its activity.

The results of investigations in NBCCS patients indicate that: 1) chromosomal instability and cellular UV hypersensitivity are not distinctive and constant features of NBCCS; 2) the clinical radiosensitivity of NBCCS is not related to increased cellular mutability.

DNA repair investigations in TTD patients, confirmed that TTD cells may have normal or enhanced UV sensitivity. Among the patients showing the DNA repair defect, different degrees of alterations in UDS level were observed. Complementation analysis indicated that the genetic defect in repair-deficient TTD cells was always the same, i. e. the XP-D mutation. The concurrence of TTD/XP-D mutation in several unrelated patients indicates that the association cannot be considered fortuitous.

IV. Objectives for the next reporting period:

The analysis of nuclear proteins modified by ADP-ribosylation will be performed in rat liver nuclei. The possible role of ADPRT in the aging process will be studied in lymphocytes from patients with premature aging syndromes. A screening for human antibodies against ADPRT will be conducted in sera of patients affected by autoimmune dieases.

In order to localize on human chromosomes the genes able to complement the repair defect in the UV^S CHO mutants representing two new groups, the characterization for human chromosome content and UV sensitivity of hybrids between these mutants and human lymphocytes will be continued. In order to clarify the genetic basis of the TTD/XP-D association, cellular and genetic investigations will be extended to other TTD patients.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1) A. Sarasin and M. Mezzina , Institut de Recherche Scientifiques sur le Cancer, Villejuif (France).

2) C.F.Cesarone, Istituto di Fisiologia Gnenerale, Università di Genova (Italy).

3) A.R. Lehmann, MRC Cell Mutation Unit, University of Sussex, Brighton, U.K.

VI. Publications:

Publications in Scientific Journals

1) Cesarone C.F., Scovassi A.I., Scarabelli L., Orunesu M. and Bertazzoni U. (1988) Depletion of adenosine diphosphate-ribosyl transferase activity in rat liver during exposure to N-2-acetilaminofluorene. Effect of thiols. Cancer Research 48: 3581-3585

2) Casoli C., Magnani G., Scovassi I., Bertazzoni U. and Starcich R. (1988) Prognostic significance of adenosine deaminase determinations in subjects with the lymphoadenopathy syndrome. J. Medical Virology 24: 413-422.

3) Lori F., Scovassi A.I., Brusamolino F., Casoli C., and Starcich R. (1988) Different distribution of DNA polymerases α and β in bone marrow and peripheral blood from human leukemia. Med. Oncol. and Tumor Pharmacother. 5: 181-186

4) Stefanini M., Lagomarsini P., Berardesca E., Borroni G., Rabbiosi G. and Nuzzo F. (1988) Normal sensititvity to mutagens, spontaneous chromosome breakage and mutation frequency in nevoid basal cell carcinoma syndrome. Arch. Dermat. Res., 280 (Suppl.): S19-S23.

5) Lehmann A.R., Arlett C.F., Broughton B.C., Harcourt S.A., Steingrimsdottir H., Stefanini M., Taylor A.M.R., Natarajan A.T., Green S., King M.D., McKie R.M., Stephenson J.B.P. and Tolmie J.L. (1988) Trichothiodystrophy: a human DNA-repair disorder with heterogeneity in the cellular response to ultraviolet light. Cancer Res.,vol. 48.

6) Stefanini M. (1988) Chromosomal mapping of human DNA repair genes. In DNA damage and repair. Castellani A. ed., Plenum Press, New York, pp. 43-49.

Short Communications

1) Bertazzoni U., Scovassi A.I., Izzo R., Colombo I., Scarabelli L. and Cesarone C.F. (1988) ADP-ribosylation of nuclear proteins during the carcinogenic process induced in rat liver by 2-AAF (1988) NATO Adv. Res. Workshop on DNA repair Mechanisms and their Biological Implications in Mammalian Cells, Fontevraud (France), October 2-7.

2) Cesarone C.F., Scarabelli L., Bertazzoni U., Scovassi A.I., Giannoni P. and Orunesu M. (1988) Changes in poly (ADP-ribose) transferase activity during rat liver regeneration. XV Riunione Soc. Ital. Fisiologia, Firenze (Italy), May 25-27.

3) Mezzina M., Nardelli J., Nocentini S., Bertazzoni U. and Sarasin A. (1988) DNA ligase activity in human cells from normal donors and from patients with Bloom's Syndrome and Fanconi's Anemia. NATO Adv. Res. Workshop on DNA repair Mechanisms and their Biological Implications in Mammalian Cells, Fontevraud (France), October 2-7.

4) Lehmann A.R., Arlett C.F., Stefanini M. and Mitchell D. (1988) Heterogeneous UV response of cells from patients with trichothiodystrophy. NATO Adv. Res. Workshop on DNA Repair Mechanisms and their Biological Implications in Mammalian Cells, Fontevraud (France), October 2-7.

5) Stefanini M., Mondello C., Riboni R., Tessera M.L. and Nuzzo F. (1988) UV sensitive Chinese hamster mutants: identification of two new complementation groups. NATO Adv. Res. Workshop on DNA Repair Mechanisms and their Biological Implications in Mammalian Cells, Fontevraud (France), October 2-7.

6) Stefanini M., Lagomarsini P. and Nuzzo F. (1988) Genetic analysis in TTD repair deficient cells confirms the occurrence of XP-D mutaion in unrelated patients. NATO Adv. Res. Workshop on DNA Repair Mechanisms, Fontevraud (France), October 2-7.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Universität - GSH Essen
Universitätsstr. 2
D - 4300 Essen 1

Contract no.: BI6-E-223-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. G. Obe
Universität - GSH Essen
Universitätsstr. 5
D - 4300 Essen 1

Telephone number: 0201 183-1 2100

Title of the research contract:

The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

List of projects:

1. The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

Title of the project no.: B16-E-223-D

The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

Head(s) of project:

Prof. Dr. G. Obe
Universität GHS Essen
Universitätsstraße 5

D - 4300 Essen 1
Scientific staff:

I. Objectives of the project:

To irradiate blood in vitro with low doses of x-rays and to examine the lymphocytes in metaphase for radiation induced chromosome aberrations. The primary objective is to verify the existence of any low dose plateau in response over the range zero to a few tens of milligrays. Blood from 20 donors will be used because variations in sensitivity of donors may influence the low dose response. All cells containing exchange type aberrations will be photographed and karyotyped in order to determine whether certain chromosomes are specifically involved in such aberrations.

II. Objectives for the reporting period:

1. To complete scoring the material at 0 and 30 mGy
2. To decode and collate the results from the 6 collaborating laboratories
3. To analyse the data for:
   a) interlaboratory variation
   b) comparison of yields at 0 and 30 mGy
   c) possible donor variability
   d) multiply damaged cells
   e) karyotyping cells with exchange aberrations

III. Progress achieved:

Methodology

Standard difference tests were applied to the data to determine the objectives a-c in section 2.

Results

Lab.	Dose (mGy)	No. Cells scored	Dicentrics	C.Rings	Excess Acentrics
1	0	10500	14	1	63
	30	10200	3	3	66
2	0	10000	9	0	2
	30	10000	11	1	8
3	0	10015	10	0	28
	30	9980	23	2	39
4	0	10000	5	0	16
	30	10000	11	1	17
5	0	9797	8	0	16
	30	9718	16	0	34
6	0	10000	5	0	9
	30	10000	10	0	26
Total	0	60312	51	1	134
	30	59898	74	7	190

The pooled donor results from the 6 laboratories

Discussion

As is usually experienced in collaborative projects some inter-laboratory differences in results are apparent. This is especially evident for the acentric aberrations reported by laboratories 1 and 2. We have found no particular explanation for this divergence. The total numbers of dicentrics scored by each laboratory are reasonably consistent but laboratory 1 is exceptional in having found significantly more dicentrics in the controls than in the irradiated cells. This is mainly due to very low yield at 30 mGy but the zero dose yield is a little high. The latter includes one cell containing 3 dicentrics from donor F (see later).

For acentric aberrations the inter-laboratory variations tend to cancel out and the total results are approximately consistent with a linear extrapolation of published dose response data. For dicentrics the background yield is consistent with previous reports of approximately 1 per 1000 cells. The yield in the irradiated cells however is low when compared with the data reported by this group of callaborating laboratories in a previous experiment (see section 6) and by extrapolating other published higher dose data assuming the linear quadratic model. Even if one excludes the odd result from laboratory 1 the yield is still low but tests show that the significance is marginal. These data therefore tend to support the idea of a threshold or plateau effect in the very low dose respone.

A test on the distributions of the control and exposed sets of dicentric data indicated no significant deviation from the Poisson distribution (zero dose $\sigma^2/y = 1.03 \pm 0.3$, 30 mGy $\sigma^2/y = 1.13 \pm 0.3$). This suggests that if inhomogeneity exists within this group of 20 subjects it could only be detected by many more cells being scored. There is a suggestion from the data that subjects with a low control yield also exhibited a low irradiated yield and conversely a high control is associated with a high irradiated yield. However this trend is not quite significant but will be subject to further testing when data from two more doses become available.

Three multiply damaged (>1 dicentric) control cells were observed; one in each of donors F, H and J, and one irradiated cell in donor N. This is a similar finding to the previous experiment (section 6) and is again significantly greater than expected from Poisson statistics.

Karyotyping the cells containing exchange aberrations is still in progress but no result is yet available.

Because of the anomalies and variations described above it was decided to score the other cells irradiated at 5 and 300 mGy that were prepared at the same time. The slides have been distributed to the collaborating laboratories and scoring is in progress.

IV. Objectives for the next reporting period:

To complete scoring the material irradiated at 5 and 300 mGy. To collate those results with the zero and 30 mGy data reported here. To analyse the full data in accordance with the objectives as described in sections 1 and 2 and to compare the results with those of a previous experiment now published (section 6).

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. State University of Leiden, Netherlands (Prof. A. Natarajan)
2. Free University of Berlin, Germany (Prof. G. Obe), in the meantime transferred to the University of Essen)
3. CEN/SCK Mol, Belgium (Dr. A. Leonard)
4. BNFL, Sellafield, UK (Dr. J. Tawn)
5. University of Rome, Italy (Dr. F. Palitti)

VI. Publications:

D.C. Lloyd, A.A. Edwards, A. Leonard, G.L. Deknudt, A. Natarajan, G. Obe, F. Palitti, C. Tanzarella, E.J. Tawn. Frequencies of Chromosomal Aberrations Induced in Human Blood Lymphocytes by Low Doses of X-Rays. Int. J. Radiat. Biol. 53 (1) 49-55, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-186-I

Università di Roma "La Sapienza"
P. le Aldo Moro, 5
I - 00185 Roma

Head(s) of research team(s) [name(s) and address(es)]:

Prof. G. Olivieri
Dip. di Genetica e Biol. molecolare
Università di Roma "La Sapienza"
P. le Aldo Moro 5
I - 00185 Roma

Telephone number: 49.56.205

Title of the research contract:

Adaptive response to low doses of radiation: studies in human cells of a possible radiation-stimulated repair.

List of projects:

1. Study of the effect of a low pre-dose of radiation on the level of chromosomal damage induced by a subsequent acute dose in human cells.

Title of the project no.: B16-E-186-I

Study of the effect of a low pre-dose of radiation on the level of chromosomal damage induced by a subsequent acute dose in human cells.

Head(s) of project:

Prof. Gregorio OLIVIERI

Scientific staff:

F. PELLICCIA, A. MICHELI, A. BOSI, S. PIETROSANTI, C. TRINCHESE

I. Objectives of the project:

To study the mechanism by which low levels of chronic radiation can trigger or induce increased repair of radiation-induced chromosome breaks (Olivieri et al., 1984).

II. Objectives for the reporting period:

To study the individual variability of human lymphocytes in induction of "adaptive response".

III. Progress achieved:

METHODOLOGY

The experiments consisted of first exposing cultured human lymphocytes to adapting doses of ^{3}H dThd or 0.01 Gy of X rays, and, subsequently challenging the cells with high doses of X rays. The cells were scored to see whether the prior exposure reduced the number of chromatid and isochromatid breaks induced by the challenging doses.

Experiments were carried out using cultures of blood from donors of different sexes. Whole blood (0.5 ml) was added to 4.5 ml of RPMI 1640 medium without fetal calf serum (Wolff et al., 1984), 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 2% phytohemmaglutinin M (Gibco).

For practical reasons 5 experiments were done in batches of 4-6 donors over a period of one year.

Blood withdrawal and seeding were performed between 8 and 9 a.m.. In females the withdrawals were limited to the first 12 days after the beginning of menses. Two different types of conditioning pretreatment were used: a) 0.01 uCi/ml of ^{3}H dThd (spec.act. 78.8 Ci/mmol) present in the cultures from 32 h after stimulation until fixation; b) 0.01 Gy of X-rays admimnistered 32 h after stimulation. The cells were subsequently challenged with 0.75 Gy of X-rays at 48 h and fixed at 50 h after stimulation. In Exp. I doses of 0.50 of 0.25 Gy have also been used. Irradiation was carried out with 200 kVp X-rays (Gilardoni MLG 200/8D,0.2 mm Cu added filtration, 8 mA, 0.60 Gy/min). Two hours before fixation, 0.1 ml of Colcemid (final concentration 2×10^{-7} M) was added to each culture, and fixation was performed according to standard cytological procedures; for each point examined two parallel cultures were set up.

RESULTS AND DISCUSSION

Human lymphocytes exposed to low doses of ionizing radiations from incorporated tritiated thymidine or from X-rays, become less susceptible to the induction of chromatid aberrations by high doses of X-rays. This indicates that low doses of ionizing radiation can produce an effect similar to the

adaptive response (A.R.) observed with alkylating agents in prokaryotes, animal and plant cells. To determine whether there is individual variability in the adaptive response to ionizing radiations we exposed human lymphocytes from 18 different healthy donors to "adapting" doses of ^{3}H dThd (0.01 uCi/ml) or X-rays (0.01 Gy) and subsequently to a "challenge" treatment of 0.75 Gy of X-rays delivered 2 h before fixation.

Four of the 18 donors did not show an adaptive response; in some cases in these individuals a synergistic response of increased, rather than decreased, damage was found. Two of these four donors showed no adaptive response to the ionizing radiations which might be, at least in part, genetically determined.

The chromosome aberrations scored in the present work have been induced in the G2 phase of the cell cycle. Because irradiation in G2 causes an A.R. comparable to that produced by irradiation in S (Olivieri et al., 1984), we chose to deliver the challenging dose in G2 to obtain a sample of metaphases somewhat more homogeneous than a sample irradiated in the S phase. However, we observed a remarkable difference between individuals in the frequency of induced aberrations. In any case the variability in the frequency of aberrations is not related with the variability in the A.R.. Thus, the absence of an A.R. has been observed in cases with low, intermediate or high frequencies of aberrations.

The variability in the A.R. does not seem to involve the level of the A.R., but rather the presence or the absence of this phenomenon.

IV. Objectives for the next reporting period:

To study whether the individual variability of human lymphocytes in induction of "adaptive response" (Bosi and Olivieri, 1988) depend on transient physiological parameters or has a genetic basis.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

S. WOLFF, J.K. WIENCKE, V. AFZAL
Laboratory of Radiobiology, University of California, San Francisco CA 94143 USA.

VI. Publications:

S. Wolff, V. Afzal, J.K. Wiencke, G. Olivieri and A. Micheli: (1988) "Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiations as well as to chemical mutagens that induce doublestrand breaks in DNA" Int. Rad. Biol. 53: 39-48.

Pelliccia F., Bosi A., Belloni G., Micheli A. and Olivieri G. 1988: "Studies on chromosome abnations induced by incorporated tritium: effects of post-treatment with hydrxyurea and caffeine in G_2 Mut Res 199: 139-144.

Bosi A., G. Olivieri Variability of the adaptive response to ionizing radiations in humans, 1988 Mut. Res. in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Consiglio Nazionale
delle Ricerche
Piazzale Aldo Moro, 7
I - 00185 Roma

Contract no.: BI6-E-171-I

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Palitti
Dip. di Genetica e Biol. Molecolare
Università di Roma "La Sapienza"
Centro di Genetica Evoluzionistica
I - 00185 Roma

Telephone number: 06-4956205

Title of the research contract:

Evaluation of the frequencies of chromosomal aberrations, induced in human blood lymphocytes by low doses of X rays (1-10 rad).

List of projects:

1. Collaboration in a joint project on the accurate estimation of dose effect relationship for chromosome aberrations induced in human lymphocytes at low doses of X rays.

Title of the project no.: Collaboration in a joint project on the accurate estimation of dose effect relationship for chromosome aberration induced in human lymphocytes at low doses of x-rays.

Head(s) of project: Prof. F. Palitti

Scientific staff: Prof.C.Tanzarella,Dr.F.Degrassi,Dr.R.De Salvia,Mr.M.Fiore and Mrs.S.Polani.

I. Objectives of the project: To irradiate blood in vitro to low doses of x-rays and to examine the lymphocytes in metaphase for radiation induced chromosome aberrations. The primary objective is to verify existence of any low dose plateau in response over the range zero to a few tens of milligrays. Blood from 20 donors will be used because variations in sensitivity of donors may influence the low dose response. All cells containing exchange type aberrations will be photographed and karyotyped in order to determine wether certain chromosomes are specifically involved in such aberrations.

II. Objectives for the reporting period:

1. To complete scoring the material at 0 and 30 mGy
2. To decode and collate the results from the 6 collaborating laboratories;
3. To analyse the data for:
   a) interlaboratory variation
   b) comparison of yields at 0 and 30 mGy
   c) multiple damaged cells
   d) possible donor variability
   e) Karyotyping cells with exchanges aberrations

III. Progress achieved:

Methodology.

Standard differences tests were applied to the data to determine the objectives a,b and d in section 2.

Results.

Lab.	Dose (mGy)	No.Cells scored	Dicentrics	C.Rings	Excess Acentrics
1	0	105000	14	1	63
	30	10200	3	3	66
2	0	10000	9	0	2
	30	10000	11	1	8
3	0	10015	10	0	28
	30	9980	23	2	39
4	0	10000	5	0	16
	30	10000	11	1	17
5	0	9797	8	0	16
	30	9718	16	0	34
6	0	10000	5	0	9
	30	10000	10	0	26
	0	60312	51	1	134
Total	30	59898	74	7	190

The pooled donor results from the 6 laboratories.

Discussion.

As is usually experienced in collaborative projects some inter-laboratory differences in result are apparent.This is especially evident for the acentric aberrations reported by laboratories 1 and 2. We have found no particular explanation for this divergence. The total number of dicentrics scored by each laboratory are reasonably consistent but laboratori 1 is exceptional in having found significantly more dicentric in the controls than in irradiated cells. This is mainly due to the very low yield at 30 mGy but the zero dose yield is a little high. The latter includes one cell containing 3 dicentric from one

donor.

For acentric aberrations the inter-laboratory variations tend to cancel out and the total results are approximately consistent with a linear extrapolation of published dose response data. For dicentric the background yield is consistent with previous reports of approximately 1 per 1000 cells. The yield in the irradiated cells however is low when compared with the data reported by this group of collaborating laboratories in a previous experiment (see section 6) and by extrapolating other published data assuming the linear quadratic model. Even if one excludes the odd result from laboratory 1 the yield is still low but tests show the significance is marginal. These data therefore tend to support the idea of a threshold or plateau effect in the very low dose response.

A test on the distributions of the control and exposed sets of dicentric data indicated no significant deviation from the Poisson distribution (zero dose $\sigma^2/Y=1.03\pm0.3$, 30 mGy $\sigma^2/Y=1.13\pm 0.3$). This suggests that if inhomogeneity exists within this group of 20 subjects it could only be detected by many more cells being scored.
From the data there is a suggestion that subjects with a low control yield also exhibited a low irradiated yield and conversely a high control is associated with a high irradiated yield. However this trend is not quite significant but will be subjected to futher testing when data from more two doses become avaible.

Three multiple damage (>1 dicentric) control cells were observed; one in each of three donors, and one irradiated cell in another donor. This is a similar finding to the previous experiment (section 6) and is again significantly greater than expected from Poisson statistics.

Karyotyping the cells containing exchange aberrations is still in progress but no result is yet avaible.

Because of anomalies and variations described above it was resolved to score the other cells irradiated at 5 and 300 mGy that were prepeared at the same time. The slides have been distributed to the collaborating laboratories and scoring is in progress.

IV. Objectives for the next reporting period:

To complete scoring the material irradiated at 5 and 300 mGy. To collate those results with the zero and 30 mGy data reported here. To analyse the full data in accordance wioth the objectives as described in section 1 and 2 and to compare the results with those of a previous experiment now published(section 6).

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1 State Univercity of Leiden,Netherlands (Prof.A.T.Natarajan)
2 Free University of Berlin,Germany,(Prof.G.Obe)
3 CEN/SCK Mol,Belgium (Dr.Leonard)
4 BNFL,Sellafield,UK (Dr.J.Tawn)
5 NRPB,Chilton,U.K. (Dr.D.Lloyd)

VI. Publications:

D.C. Lloyd,A.A.Edwards,A.Leonard,G.L.Deknudt,A.T.Natarajan,G.Obe, F.Palitti,C.Tanzarella,E.J.Tawn. Frequencies of chromosomal aberrations induced in human blood lymphocytes by low Doses of x-Rays.
Int.J. Radiation Biol. 53(1) 49-55;1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université Paris VII
Institut Jacques Monod
Place Jussieu, 2
F - 75251 Paris Cédex 05

Contract no.: BI6-E-154-F

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. M. Radman
Institut Jacques Monod
Université Paris VII
Place Jussieu, 2
F - 75251 Paris Cédex 05

Telephone number: 01-336.25.25

Title of the research contract:

Molecular basis of radiation-induced mutagenesis from bacteria to humans. New Experimental Systems.

List of projects:

1. Molecular basis of radiation-induced mutagenesis from bacteria to humans. New Experimental Systems.

Title of the project no.: MOLECULAR BASIS OF RADIATION INDUCED MUTAGENESIS IN PROKARYOTES AND EUKARYOTES : NEW EXPERIMENTAL SYSTEMS.

Head(s) of project: Prof. M. RADMAN - Laboratoire de Mutagénèse
INSTITUT JACQUES MONOD - C.N.R.S./Université Paris 7
Tour 43 - 2, Place Jussieu
F-75251 PARIS CEDEX 05, France

Scientific staff:
Dr P. BROOKS, Dr P. DESCHAVANNE, Ms C. DOHET, Dr M. PETRANOVIC, Ms M.P. DOUTRIAUX, Ms S. DZIDIC, Dr R. RAYSSIGUIER, Dr R. WAGNER, M. LANGE, F. PIONNEAU, I. VARLET.

I. Objectives of the project:

(a) Physical mapping and quantification of mutations in any DNA : isolation of mismatch-recognizing proteins and the study of structures of diverse DNA base-pair mismatches in defined oligonucleotides.

(b) Molecular mechanisms and genetic control of radiation-induced mutagenesis through misreplication of DNA lesions and radiation-stimulated gene conversion.

II. Objectives for the reporting period:

(i) Defining the role of general mismatch repair system in the specificity of genetic recombination : a new clue to the nature of chromosomal rearrangements.

(ii) Mismatch repair mechanisms in vertebrates : in vitro studies using frog egg extracts

(iii) Enzymes involved in misreplication of a common (also radiation-induced) DNA lesion : abasic site.

III. Progress achieved:

1. Methodology.

Diverse genetic manipulations of bacteria Escherichia coli and Salmonella typhimurium and their bacteriophages, lambda, M13 and P22. DNA strand separations, heteroduplex formation in vitro, transfection, oligonucleotide mutagenesis, DNA methylations in vitro, in vitro tests for mismatch repair, physical mapping of mismatch repair tracts, inter-species recombination, sequencing gels, purification of DNA polymerases.

2. Results et Discussion.

In each area of initial objectives, we have had a real breakthrough : in (i), an unexpected importance of the mismatch repair on recombination between species, chromosomal stability and evolution of species is now evident ; this discovery in bacteria was already largely confirmed by our in vitro studies with frog egg extracts, and in (ii), after over a dozen years of our CEC-supported research efforts, we have identified (in collaboration with Dr M. Goodman's group) a SOS-inducible error-prone DNA polymerase (DNA polII of E. coli). In particular :

(i)/1. After having identified the mismatch-stimulated anti-recombination activity of the general mismatch repair system in vivo, we have demonstrated that this repair system alone prevents recombination between "homeologous" DNA sequences (which are about 80% homologous sequences, such as those of related species or different copies of the same repetitive sequence family). This discovery offers the basis for the understanding of the role of repetitive sequences in mammalian genomes, for the origin and mechanism of chromosomal aberrations and opens a new area of in vivo genetic manipulation, including "gene therapy".

(i)/2. The sequence context effect on efficiency of mismatch repair was identified: repair is proportional to the G:C content of the close mismatch environment.

(ii)/1. In vitro repair of G:T, A:C and +/-2 base mismatches has been demonstrated in Xenopus laevis egg extracts. Repair tract is long (from about a hundred up to a thousand bases), as in bacteria. Cytosine methylation does not seem to direct mismatch repair, but nicks flanking the mismatch stimulate and direct repair to the nicked strand. This result also demonstrates the mismatch-stimulated anti-recombination in a vertebrate.

(ii)/2. Repair of a mismatch mimicking 5-methylcytosine deamination (G:T) was detected only in the direction G:T to G:C, as expected for a specific correction system.

(iii). Using a novel substrate with one unique abasic site, we can quantitatively measure the lesion-bypass synthesis in sequencing gels. The assay made possible the identification and purification of an inducible DNA polymerase capable of inserting a base (usually adenine) opposite the abasic site. The purified polymerase is identical to the DNA polymerase II of E. coli. The genetics of this obscure enzyme is in progress.

IV. Objectives for the next reporting period:

(a) Genetic and physical studies of intergeneric recombinants

(b) Study of the effect of multiple mut mutants on heterospecific recombination to reveal some aspects of the interaction of Mut proteins in mismatch repair.

(c) Continued characterization of mismatch repair in vitro with Xenopus eggs and human cells and in vivo with Xenopus oocytes.

(d) Use of mismatch repair systems for detection of human mutation.

(e) Demonstration in vitro of mismatch-dependent antirecombination.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr A. Brandenburger, Mrs F. Laengle-Rouault and Dr G. Maenhaut-Michel (Département de Biologie Moléculaire, Université Libre de Bruxelles - 1640 RHODE ST GENESE, Belgium)

Dr V. Fazakerley and W. Guschlbauer (Service de Biochimie) - Centre d' Energie Atomique, Bât. 142 C.E.N. Saclay - 91191 GIF sur YVETTE Cedex

VI. Publications:

1. Radman M. (1988) : "DNA repair and genetic alterations". In "New Trends in Genetic Risk Assessment". G. Jolles and A. Cordier Eds. (Academic Press Publ.), in press.

2. Radman M. (1988) : "Mismatch repair and genetic recombination". In : "Genetic recombination", eds. R. Kucherlapati and G.R. Smith. "Mismatch repair and genetic recombination". American Soc. of Microbiol. pp. 169-182.

3. Radman M. and R. Wagner (1988) : "High fidelity of DNA replication". Scientific American 259, 40-47.

4. Quinto I., Tenenbaum L. and M. Radman (1988) : "Genotoxicity profile of monofunctional alkylating agents in E. coli : quantitative correlations with carcinogenic potency in rotends". Proc. Natl. Acad. Sci. USA, in press.

5. Brooks P., Dohet C., Petranovic M. and M. Radman (1988) : "Mismatch repair in Escherichia coli and in Xenopus egg extracts". In :Mechanisms

and consequences of DNA damage processing". Friedberg E.C. and Hanawalt P. eds., Alan Liss Publ., pp. 167-171.

6. Dzidic S. and M. Radman (1988) : "Genetic requirements for hyperecombination by very short patch mismatch repair : involvement of E. coli DNA polymerase I". Mol. Gen. Genetics, in press.

7. Bonner C.A., Randall S.K., Rayssiguier C., Radman M., Eritja R., Kaplan B.E., Mc Entee K. and M. Goodman (1988) : "Purification and characterization of an inducible Escherichia coli DNA polymerase capable of insertion and bypass at abasic lesions in DNA". J. Biol. Chem., 263, n° 35, 18946-18952.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université Libre de Bruxelles
avenue Fr. Roosevelt, 50
B - 1050 Bruxelles

Contract no.: BI6-E-155-B

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. M. Radman
Lab. de Biophysique et Radiobiol.
Université Libre de Bruxelles
rue des Chevaux, 67
B - 1640 Rhode St Genèse

Prof. J. Roumelaere
Lab.Biophysique & Radiobiol.
Université Libre de
Bruxelles
rue des Chevaux 67
B - 1640 Rhode St. Genèse

Telephone number: 02-358.35.30 (Ext. 226)

Title of the research contract:

Mutagenic effects of ionizing radiation in bacteria and mammalian cells.

List of projects:

1. Molecular specificity of radiation-induced genetic alterations in E. coli : risk assessment for humans.

Title of the project no.: BI6-E-155-B

MOLECULAR SPECIFICITY OF RADIATION-INDUCED GENETIC ALTERATIONS IN E.COLI : RISK ASSESSMENT FOR HUMANS

Head(s) of project: M. RADMAN and MAENHAUT-MICHEL, G.

Scientific staff: P. CAILLET-FAUQUET
G. MAENHAUT-MICHEL

I. Objectives of the project:

This project aims at studying the mechanisms and regulation of mutagenic processes and in particular the SOS mechanism which is triggered by ionizing radiations and radiomimetic chemicals in bacteria. The correlation between the spectrum of induced mutations and different DNA damaging agents has been studied extensively in several laboratories these last years. But the molecular mechanism of mutagenesis is not yet elucidated. The mutation spectra induced in human cells by different DNA damaging agents that provoke mutations via the SOS mechanism in E.coli bacteria, showed close similarities with the spectra obtained in E.coli. This may mean that basically similar mutagenic mechanisms operate in most organisms and encouraged us to consider E.coli as a pertinent model and a convenient tool to study the mechanisms of mutagenesis.

II. Objectives for the reporting period:

The objectives for the reporting period was to study the conversion of premutagenic lesions in mutations, in specific sequences of the DNA. Mutations are not randomly distributed along DNA. Several factors govern the conversion of premutagenic lesions into mutations among them, reactivity of DNA bases, sequence context and local configuration of the DNA helix. The identification of E.coli proteins involved in mutagenesis and able to recognize such specific local configuration was initiated.

III. Progress achieved:

MOLECULAR SPECIFICITY OF RADIATION-INDUCED GENETIC ALTERATIONS IN E.COLI : RISK ASSESSMENT FOR HUMANS

Chemical and physical (UV light, X or gamma rays) mutagens damage DNA by covalently altering its chemical structure, generating a premutagenic lesion. Only a small fraction of the premutagenic lesions are converted into mutations. For non-informative lesions this conversion involves the induction of a set of coregulated genes (i.e. the SOS response in E.coli). In the case of most mutagens, mutations are generally not distributed randomly along DNA but are found to be clustered within specific sequences referred to as mutation hot spots. The correlation between mutation hot spots and DNA sequences has been extensively studied for a chemical carcinogen belonging to the family of aromatic amines : the N-2-acetyl-aminofluorene (AAF)[1]. More than 90% of mutations induced by AAF adducts are frameshift mutations whose induction depends upon UV-irradiation of the bacteria (for SOS induction). These mutations appear either within monotonous runs of guanine residues (repetitive sequences) or within short stretches of alternating GC base pairs. The mechanism inducing mutations in repetitive sequences involves the participation of umuCD gene products and RecA protein whereas the mechanism inducing mutation in alternating GC sequences does not. SOS dependent but umuCD independent mutagenesis was also induced by UV and gamma radiation[3]. A strategy to study this specific pathway of SOS mutagenesis was developed involving the search for new mutants of E.coli, suppressing specifically this type of mutagenesis.

1. Methodology

A genetic tool for the screening of a collection of E.coli mutants was developed in collaboration with the Laboratory of Dr. Fuchs (Institut de Biologie Moléculaire et Cellulaire, IBMC, CNRS, Strasbourg, France). A plasmid pX2 derived from pBR322 was constructed carrying a specific target for AAF-induced mutagenesis at alternating GC sequences. This new plasmid confers a tetracycline sensitive phenotype to bacteria. The reversion to the wild type tetracycline resistant phenotype is obtained by a deletion of two alternating GC base pairs. This reversion was shown to be efficiently induced by the ultimate carcinogen N-Aco-AAF.
A strain of E.coli carrying a defective mutation in the LexA repressor (lexA(Def)) which allows constitutive expression of the SOS genes was transformed with the plasmid pX2. A culture of this strain was mutagenized with UV and then screened for detection of mutants that are unmutable by N-Aco-AAF.

2. Results and discussion

Several mutants were isolated in this way ; they were mapped by Hfr crosses, P1 transduction and complementation with F' episomes. One of them is thermosensitive, maps between 63' and 68' and is sensitive to UV. It is suggested that it could be in the primase, i.e. in dnaG gene. Further precise mapping is under investigation.
Another mutant was found to map in the recA gene and to affect the recombination properties of the RecA protein. Genetic analyses have shown that RecA protein is not necessary for the conversion of premutagenic lesions into mutations in alternating GC sequences if the LexA repressor is destroyed by a mutation in the lexA gene (lexA Def)[2]. However, the isolation of a lexA(Def)recA mutant that prevents AAF mutagenesis at

alternating GC sequences suggests that RecA protein might prevent mutation fixation by interfering with the mutagenic process that converts modified guanine into mutations. Further analysis of the specific interactions between RecA protein and alternating GC sequences will be done. A thirth mutant has been isolated and characterized that suppresses AAF-induced frameshift mutagenesis both in alternating GC sequences and repetitive GGG sequences. UV-induced base substitution mutagenesis is normally induced in this mutant which is not sensitive to UV. This mutation maps near the purE gene. Further characteirzation will be done involving gene cloning and identification of the proteins.

(1) Fuchs, R.P.P. (1983) J. Mol. Biol. 177 : 173-180.

(2) Koffel-Schwartz, N. and Fuchs, R.P.P., Molec. Gen. Genet., in press.

(3) Maenhaut-Michel, G. and Caillet-Fauquet, P. (1984) J. Mol. Biol. 177 : 181-187.

IV. Objectives for the next reporting period:

The mutated RecA protein will be purified from the mutant that has been isolated (see above) and the interaction of this protein with alternating GC sequences will be studied in collaboration with the Laboratory of R. Fuchs (IBMC, Strasbourg).
On the other hand, the isolation of new mutants will be pursued in view to identify new SOS genes involved in umuC-independent mutagenesis.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Groupe de cancérogénèse et de mutagénèse moléculaire et structurale, 15, rue René Descartes, 67084 Strasbourg Cedex (France)
R.P.P. Fuchs Directeur de Recherche
R. Bintz Doctorant

VI. Publications:

Maenhaut-Michel, G: (1988) Genetic characterization of the SOS mutator effect in E. coli
In: "DNA replication and mutagenesis" ed. R.E. Moses and W.C. Summers American Soc. for Microbiol. Washington D.C. 20006.

Caillet-Fauquet,P. and Maenhaut-Michel, G : (1988) Nature of the SOS mutator activity: Genetic characterization of untargeted mutagenesis in Escherichia coli. Mol. gen. Genet., 213: 491-498.

Maenhaut-Michel, G. and Caillet-Fauquet, P.
Genetic characterization of SOS-induced untargeted mutagenesis of phages (Abstract) (1988) J. Cell. Biochemistry suppl. 12A 334.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre National de la
Recherche Scientifique
Quai A. France, 15
F - 75700 Paris

Contract no.: BI6-E-163-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Sarasin
Institut de Recherches
Scientifiques sur le Cancer
B.P. n° 8
F - 94802 Villejuif Cédex

Telephone number: 01-47.26.46.58

Title of the research contract:

Molecular analysis of mutagenesis in mammalian cells treated by radiations and chemical carcinogens.

List of projects:

1. Use of specific genes as probes for mutagenesis, oncogenesis in xeroderma pigmentosum patients, and characterization of DNA ligase activities.

Title of the project no.: B16 - 163 - F

Molecular analysis of mutagenesis in mammalian cells treated by radiations and chemical carcinogens.
Use of specific genes as probes for mutagenesis, oncogenesis in xeroderma pigmentosum patients, and characterization of DNA ligase activities in human cells.

Head(s) of project:

Dr. Alain SARASIN
Laboratory of Molecular Genetics
Institut de Recherches Scientifiques sur le Cancer
B.P. n° 8 - 94802 - VILLEJUIF CEDEX (France)

Scientific staff:

Alain SARASIN, Alain GENTIL, Mauro MEZZINA, François BOURRE, Leela GROSJEAN, Michael R. JAMES, Catherine MADZAK, Anne STARY, Anne LICHTENBERGER, Horacio G. SUAREZ.

I. Objectives of the project:

a) Characterization at the molecular level of the type of mutation induced by UV-light or by different chemical carcinogens in mammalian cells : development of modified SV40 vectors and of several shuttle vectors.

b) Analysis of oncogenesis in the xeroderma pigmentosum disease.

c) Characterization of DNA ligase(s) in mammalian cells.

II. Objectives for the reporting period:

a) Mutagenic efficiency of UV-induced DNA lesions.

b) Development of new shuttle vectors.

c) Oncogene activation in xeroderma pigmentosum tumors.

d) Analysis of DNA ligase in some human cancer-prone diseases.

III. Progress achieved:

a) Mutagenic efficiency of UV-induced DNA lesions.

UV-light essentially induces pyrimidine dimers and pyrimidine (6-4)pyrimidone lesions on DNA. By using a genetic assay based on the reversion of the temperature-sensitive phenotype of tsA58 or tsB201 SV40 mutants, we showed that pyrimidine dimers are essentially responsible for virus lethality after UV-irradiation. Both lesions are mutagenic in this assay giving rise to base substitutions opposite them. However, the mutation spectra are partially different indicating that both lesions are mutagenic but do not induce the same type of mutations. The direct comparison of the two spectra tells us the respective role of these two lesions and eventually of a new lesion we have found present on ACA sequence. Moreover mutation spectra in general were found to be sensitive to the mode of DNA transfer into the host cell. DNA lesions are more lethal and more mutagenic when virus infection is used as compared to naked DNA transfection. Some mutation hot-spots are common to the two protocols whereas other hot spots are found with only one or the other. Interestingly enough, several scattered base substitutions were found after DNA transfection while usually only one mutation was found after virus infection. Similarly, the spontaneous mutation spectra are different according to the experimental way to introduce the DNA into the cells. The spontaneous hot spots appeared to be essentially present on putative secondary structures after DNA transfection.

b) Development of new shuttle vectors.

Our laboratory has been involved in the design of new shuttle vectors for studying mutagenesis in a variety of mammalian host cells. We developed the Pi SVF 1 vector which enables us to produce the two DNA strands as single-strand species in bacteria. This permits us to study replication, repair and mutagenesis of this vector after its transfection as single-strand DNA in monkey COS cells. We showed that this single-strand DNA can be replicated in COS cells and that UV-irradiation of that DNA leads to a higher mutation frequency on the lac O or lac Z targets. Heteroduplex formation between one UV-irradiated strand and one unirradiatied strand gives rise to double-strand molecules with UV-lesions located only on one strand. We showed that such molecules can replicate in COS cells but the irradiated strand is almost completely lost during this step due to either a preferential repair or a rolling circle type of replication. This

result indicates for the first time the specific loss of the damaged strand in mammalian cells.

We were also interested in Epstein-Barr virus based shuttle vectors which replicate episomally in human cells. Since the copy number of these vectors is usually very low (between 10 and 100 per cell) we try to increase it by cloning in such vectors the SV40 replication origin. Therefore in conditions where the SV40 T antigen is expressed, this vector (p205-GTI) is replicated on the SV40 mode which should lead to a high copy number per cell. Interesting results appeared when we studied the replication of this vector in cells expressing the SV40 T antigen. Indeed, a very high level of intramolecular recombination was detected. DNA sequences of the recombinational hot spots indicated that the SV40 enhancer and promoter sequences were directly involved in the recombination sites.

c) Oncogene activation in xeroderma pigmentosum tumors.

Xeroderma pigmentosum (XP) is a hereditary disease characterized by numerous malignant epitheliomas on sun-exposed parts of the skin due to DNA repair deficiencies. We have isolated by the NIH 3T3 assay three activated oncogenes derived from three different XP tumors. Two were due to a base substitution on the 61 codon of N-ras and one was found on the 12 codon of Ki-ras. In all cases, the mutation arrived at a position where a UV-induced DNA lesion (CC or TT) can be made after sun-light exposure. More characteristically, we found that 30-40 % of XP epithelioma harbored an amplified Ha-ras. These results appeared to be either specific of XP tumors or specific of skin epithelioma. Experiments are in progress to analyze the effects of elevated Ha-ras expression in non transformed XP cells.

d) Analysis of DNA ligase in some human cancer-prone diseases.

We have analyzed qualitatively and quantitatively DNA ligase activities in cultured fibroblasts and lymphoblasts derived from normal, XP, ataxia telangiectasia, Fanconi's anemia and Bloom's syndrome (BS) patients. No significant differences were found in ligase activity between these different cell lines. However for ligase I isolated from BS cells we found it was precipitated during the polymin P precipitation step in the pellet with nucleic acids, while the enzyme from the other cells stayed in the supernatants. This divergent behaviour for the BS ligase may explain the discrepancy published in the literature with this syndrome.

IV. Objectives for the next reporting period:

a) Use of new shuttle vectors to analyze mutagenesis of a unique lesion.

b) Use of EBV shuttle vectors for regulation of gene expression in human cells.

c) Role of oncogene activation in the cancer-proneness of xeroderma pigmentosum patients.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. J.M. ROSSIGNOL, E.R. 272, Institut de Recherches Scientifiques sur le Cancer, B.P.n° 8, 94800 - VILLEJUIF (France).
Dr. Umberto BERTAZZONI, Istituto di Genetica Biochemica Evoluzionistica del C.N.R., Via Abbiategrasso n° 207 - 27100 - PAVIA (Italy).
Dr. Monique VUILLAUME, U.A. 686, E.N.S., 46, rue d'Ulm, 75230 - PARIS (France)

VI. Publications:

. **C.F.M. MENCK, M.R. JAMES, G. RENAULT and A. SARASIN**
Shuttle viruses and episomal vectors for mutagenesis and gene expression studies in monkey and human cells.
Mechanisms and consequences of DNA damage processing, UCLA, New Series, vol. 83, Editor, E. Friedberg and P. Hanawalt, Alan R. Liss, Inc., New York, NY (1988), 511-519.

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. **A. SARASIN, L. DAYA-GROSJEAN, H.G. SUAREZ, B. CHRETIEN, M.F. AVRIL**
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Médecine/Sciences, 1988, 4:643-646.

. **A. SARASIN**
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Mutation Res., 220, 1989, in press.

. **C.F.M. MENCK, C. MADZAK, G. RENAULT, A. MARGOT and A. SARASIN**
SV40-based shuttle viruses.
Mutation Res., 220, 1989, in press.

. **M.R. JAMES, A. STARY, L. DAYA-GROSJEAN, C. DROUGARD and A. SARASIN**
Comparative study of Epstein Barr virus and SV40 based shuttle-expression vectors in human repair-deficient cells.
Mutation Res., 220, 1989, in press.

. **C. MADZAK, C.F.M. MENCK, J. ARMIER and A. SARASIN**
Analysis of single-stranded DNA stability and damage-induced strand loss in mammalian cells using SV40-based shuttle vectors.
J. Mol. Biol., 1989, in press.

. **F. PUVION-DUTILLEUL and A. SARASIN**
Chromatin and nucleolar changes in xeroderma pigmentosum cells resembling aging-related nuclear event.
Mutation Res., 1989, 219:57-70.

. **H. G. SUAREZ, L. DAYA-GROSJEAN, D. SCHLAIFER, P. NARDEUX and A. SARASIN**
Activated oncogenes in human skin tumors from a repair deficient syndrome, xeroderma pigmentosum.
Cancer Research, 1989, in press.

. **A. SARASIN**
Shuttle vectors for studying mutagenesis in mammalian cells.
Photochem. and Photobiol., 1989, in press.

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DNA ligase activity in human cell lines from normal donors and Bloom's syndrome patients.
Nucl. Acid. Res., 1989, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-E-164-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.R.K. Savage
Cell and Molecular Biology Unit
Medical Research Council
Harwell, Didcot
GB - Oxon OX11 ORD

Telephone number: 0235-834393

Title of the research contract:

Analysis of cell cycle radiosensitivity in normal and mutant cells using replication banding techniques.

List of projects:

1. A study of chromosome aberration induction and DNA replication delay at different stages of the DNA synthesis phase of the cell cycle.

Title of the project no.: A Study of chromosome aberration induction and DNA replication delay at different stages of the DNA synthesis phase of the cell cycle.

Head(s) of project: Dr. J.R.K. Savage

Scientific staff: Dr. Z.S. Aghamohammadi

I. Objectives of the project: To standardise and improve the fine division of the cell cycle using replication-banding techniques in cultured human cells.

To use these techniques to investigate radiation effects, in particular, chromosomal aberrations. Cell-cycle perturbation and effects on chromosome replication in both normal and genetically abnormal cells.

II. Objectives for the reporting period:

a) To finalise and test the probit/probit method for analysing and comparing replication programmes.

b) To reinvestigate "localisation by default" in human cells.

c) To standardise the reverse staining method in order to examine late-replicating bands in more detail.

III. Progress achieved:

Methodology

1. During DNA synthesis, each pair of homologous chromosomes replicates its bands in a precise order and at a specific time. When using asynchronous cell populations, this replication programme has to be reconstructed from a series of "stills" - serial samples taken at intervals through S-transit. Obviously, the result obtained is very dependent upon the kinetic progression of cells through the cycle, and any perturbation introduced into this by treatments like radiation.

A difficulty arises when we wish to compare the replication programme of a chromosome in two different cultures (differing in origin or treatment). Kinetic differences make it impossible to obtain two samples for comparision containing the same "mixture" of cells. Thus a false programme difference could be introduced or a real one masked.

We have developed a method of comparison which overcomes this problem. It is based upon the observation that, with serial sampling after the addition of BrdU to "steady state" cell cultures, the appearance curves for replication bands are sigmoidal and very well approximated by cumulative Normal distributions with very similar standard deviations.

If a family of such curves, closely spaced in time, is sampled at two times the two observed frequencies for each curve are related, their probits all lying on a single straight line. This line has a slope of 45° and is displaced from the origin by an amount related to the interval between the two samples. Given two identical families of such curves, exactly the same relationship will hold if one sample is drawn from each. If, however, the two families differ (in order, spacing, standard-deviations, etc) the probit/probit plot will deviate in various ways from a straight line with 45° slope.

Any two sub-sets of chromosome replication-band frequencies can be regarded as derived from a family of cumulative Normal curves and probit/probit plots used to test the similarity of their replication programme.

2. Based on Giemsa staining in phosphate buffer at high pH, we have standardised a method of reverse-harlequin staining (RHQ) [BrU containing chromatin stains dark, non-BrU chromatin pale] and we are now able to apply this as a routine for experimental work.

Results and Discussion

1. The probit/probit method has been applied to several problems. For example, to compare replication programmes of various chromosomes in cultured normal and ataxia telangiectasia fibroblasts; to show that the late-replicating X processes its early bands at the same rate and in in the same order as its early replicating counterpart in both lymphocytes and fibroblasts - it does not appear to speed up. (Savage & Papworth, 1988, Reddy, Savage & Papworth, 1988).

Currently we are using the method to look at the time of replication of band Xq27 in lymphocytes from fra-X patients. Later we will look at radiation-induced perturbation.

2. The RHQ staining gives a very high replication band resolution because of its sensitivity to the presence of BrU substituted chromatin. This makes it possible to employ Pulse BrdU regimes which can tell us which bands are replicating at any point in time through S.Pulses as short as 5 minutes can be detected. Such regimes offer opportunity to investigate the mid-S regions with much greater accuracy than can be done with the more usual "terminal" BrdU regimes. Claims that there are no intermediate (transition) patterns between "R" and "G" patterns have not been substantiated.

3. The fra-X work mentioned in the last report has been published. (Savage & Fitchett, 1988) Growth in low-folate conditions perturbs the cell cycle, and has led us to investigate the question of a "block", said by many to be at the G_1/S border. Using our fine cycle subdivision we found no evidence of any specific single blocking site for methotrexate. Rather, cells slowed and stopped where they were when the folate antagonist was added and continued from that position when normal folate levels were restored (Savage & Pradad, 1988).

4. Sister-chromatid Exchange (SCE) is a widely used end-point in tests for environmental mutagens. Low LET radiations do not induce SCE in resting lymphocytes, but recently we obtained evidence that 42 MeV neutrons do (Savage & Holloway, 1988, Brit. J. Radiol., 61, 231-234). The RBE is therefore undefined and effectively infinite.

We have now shown that plutonium-238 α-particles can also produce SCE (Aghamohammadi, Goodhead & Savage, 1988, Int. J. Radiat. Biol., 53, 909-915) and we are following up these findings with tests of additional radiation qualities.

IV. Objectives for the next reporting period:

1. Further develop and apply the RHQ technique to study the programme of late replicating bands with particular reference to the fra-X syndrome and radiation-induced perturbation.
2. To apply the RHQ "pulse" technique to the study of replication during mid-S with especial emphasis on the R-band/G-band transition region.
3. Continuing the work on "localisation by default", to look in more detail at the location of radiation-induced aberration break-points in relation to replication bands during S-phase transit.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

REDDY, K.S., SAVAGE, J.R.K. & PAPWORTH, D.G. (1988)

Replication kinetics of X chromosomes in fibroblasts and lymphocytes. Hum. Genet., 79, 44-48.

SAVAGE, J.R.K. & FITCHETT, M. (1988)

The behaviour of fragile X and other aberrations during recovery from low folate conditions. Chromosoma (Berl.). 96, 391-396.

SAVAGE, J.R.K. & PAPWORTH, D.G. (1988)

A method for comparing DNA replication programmes at the level of the chromosome bands. J.Theoret.Biol., 134, 365-377.

SAVAGE, J.R.K. & PRASAD, R. (1988)

Generalised blocking in S phase by methotrexate. Mutation Res., 201, 195-201.

AGHAMOHAMMADI, S.Z., GOODHEAD, D.T. & SAVAGE, J.R.K. (1988)

Induction of sister chromatid exchanges (SCE) in G_0 lymphocytes by plutonium-238 α-particles. Int.J.Radiat.Biol., 53, 909-915.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-224-GR

Greek Atomic Energy Commission
National Research Center for
Natural Sciences "Demokritos"
Aghia Paraskevi POB 60228
GR - 15310 Athens

Head(s) of research team(s) [name(s) and address(es)]:

Dr. E.G. Sideris
Rad. Mut. Project, Division Biology
N.R.C.N.S. "Demokritos"
Aghia Paraskevi POB 60228
GR - 15310 Athens

Telephone number: 301-6513111

Title of the research contract:

Radiobiological damage induced into mammalian and human cells by low energy monoenergetic protons and calculations of the RBE factors for risk estimations.

List of projects:

1. Radiobiological damage induced into mammalian and human cells by low energy monoenergetic protons and calculations of the RBE factors for risk estimations.

Title of the project no.: Radiobiological damage induced into mammalian and human cells by low energy monoenergetic protons and calculations of the RBE factors for risk estimation.

Head(s) of project: E.G. Sideris

Scientific staff: Dr. A.A. Katsanos
Dr. A. Perris
Mr. P. Pialoglou
Mr. G. Loukakis

I. Objectives of the project: Protons have attracted the interest of radiotherapists, radiologists and physicists as a radiation of potential use in the treatment of cancer mainly because of their property of having a very sharply defined effective range in the tissue. This work aims in gathering radiobiological data related to the action of ionizing radiations in conjunction to radiation quality and dose rate, the two primary factors affecting the action of ionizing radiation in biological systems.

II. Objectives for the reporting period: Study of the survival and the frequency of chromosome aberrations in cells of V-79 cell line exposed to different doses of monoenergetic protons of different energy. Estimation of the frequency of induced DNA breaks. Calculation of the corresponding RBE values and estimation of the α and β coefficients of the expected from the theory of Dual Action linear quadratic relationship. Study of the effect of the inhibition of the ADPRT on the correlation of damage between the frequency of induced chromosomal aberration and the mitotic prelifaration.

III. Progress achieved:

Methodology

The biological system which was used in this work was the V79 line from chinese hamster cells. The medium was removed before irradiation and the cells were covered with a thin Mylar foil. The cells were irradiated either with monoenergetic protons with energy 7.4 MeV (L.E.T. 5.8 KeV/ m) on cell's surface at a dose rate of 3 Gy/min or with Cobalt-60-gamma rays with a dose rate of 5 Gy/min. Immediately after irradiation the cells were trypsinised and resuspended to measure the survival ability (Perris et al., 1986) or to measure the induction of chromosomal aberrations (Sideris E.G. et al., 1984). Chromosomal aberrations were classified in dicentrics (in which tricentrics and centric rings were included also) and in excess acentrics (in which all the types of deletions not associated with the formation of dicentrics were included) (I.A.E.A., 1986). Chromatid type of aberrations were not included since it was found to consist only 2% of the total number of aberrations. Experimental data for survival were fitted, using a least square method, to the equation $S=aD+bD^2$ and the chromosomal aberrations yield to the equation $Y=c+aD+bD^2$. The obtained RBE values were estimated either by using the parameters of the fitted equations or by using the fitted dose-response curves.

Results and discussion

7.4 MeV protons have a greater efficiency than gamma rays in inducing cell killing. The R.B.E. value derived on the basis of dose response coefficinets was found to be 1.5. On the basis of D_{37}, which was estimated from the fitted curves of Figure 1, the value of $R.B.E._{37}$ was found to be 1.3. When for the estimation of the R.B.E. the mean inactivation dose, D, was used (Kelleler, A.M., 1965) the value of $R.B.E._D$ was found to be 1.7. 7.4 MeV protons induce a larger yield of aberrations, both of dicentric and of acentric type, compared to cobalt 60 gamma rays. From the coefficients of the fitted dose response equations $R.B.E._a$ was computed and in the case of dicentrics was found to be 1.0 while in the case of acentrics the value was 0.7. When mean inactivation dose was used in a similar way as for the survival curves the values of $R.B.E._D$ were estimated to be 1.3 dicentrics and 1.4 for acentrics. The value of $R.B.E._{37}$ was in the case of cells without aberrations 1.1 and the value of $R.B.E._D$ 1,15,

Since it is generally accepted that the molecule of DNA is the primary target of ionizing irradiation is expected that any damage in the genetic material of the cell will be significant for the function of cell's mitotic apparatus. Early experiments (Lea D.E., 1948) indicated that chromosome structural changes are responsible for the inactivation of Drosophila melanogaster eggs and for the inactivation of pollen grains of Tradescandia (ibid.). Some investigators (Lloyd D.C., et al., 1975, C.J.Roberts and P.D. Holt, 1982 and 1985) suggest that chromosomal aberrations are the only reason that causes cell's proliferation death. Such suggestion is based upon the similarity of survival curves with the dose response curves for cells without any aberrations and the similarity between the values of $R.B.E._{D_o}$ which was computed from these two types of curves (ibid).

Comparison of $R.B.E._{37}$ and $R.B.E._{D}$ values derived from survival curves with those derived from the fraction of cells without aberrations indicate that $R.B.E._{37}$ and $R.B.E._{D}$ obtained from survival dose-response curves are greater than the corresponding R.B.E. values which were computed for the cells without aberrations. Similarly comparison between $R.B.E._{D}$ values from survival dose response curves with $R.B.E._{D}$ values from dicentric and excess acentric yields indicates that $R.B.E._{D}$ value from survival curves is greater than the values obtained from dicentric's and acentric's induction. Therefore the type of chromosomal damage which is associated with the formation of dicentric chromosomes and acentric fragments in terms of R.B.E. is not the only type of damage which attributes to cell inactivation. In addition evidence provided by Barendsen (Barendsen, 1979 and Zoetlief and Barendsen, 1983) indicates that dicentrics and centric rings cannot be the only cause of reproductive death. This difference for R.B.E. values obtained from the end-pcint of inactivation and the end-point of chromosomal aberrations can be explained assuming that a. Other type of chromosomal damage, which is not observable in the form of dicentrics and deletions (e.g. small chromosomal rearrangements or point mutations), is also associated with cell inactivation or/ and b. There is another type of damage, not related to chromosomes, which also affects the capacity of cell to proliferate after irradiation.

IV. Objectives for the next reporting period: Continuation of the work on determining RBE values for low energy monoenergetic protons. Estimation of RBE values from DSB induction and correlation of these values to RBE values from other end points. Study of the "repair effect" through the use of repair inhibitors following exposure to monoenergetic protons. Parallel orientation work on the use of inverse gas chromatography, and γ-γ perturbed correlation method developed by our group (Kalfas et al. 1984, Int. J. Appl. Rad. Isot. 35:889-893) will be used for the study of radiation effects of monoenergetic protons.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

1. PIALOGLOU P., E.G. SIDERIS and A. PERRIS (in press) Cell Survival and chromosomal aberration frequency in V79 chinese hamster cells exposed to low energy protons Proceedings of 21st Meeting of European Society for Radiation Biology pages 001-007

2. PIALOGLOU P., E.G. SIDERIS and A. PERRIS (in press) Enhancement of cellular damage induced by gamma rays after inhibition of poly-(ADP)-ribose polymerase by 3-aminobenzamid. Proceedings of 21st Meeting of European Society for Radiation Biology pages 001-006.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB - Oxon OX11 ORQ

Contract no.: BI6-E-225-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Stather
Biomedical Effects Department
NRPB
Chilton, Didcot
GB - Oxon OX11 ORQ

Telephone number: 0235-831600

Title of the research contract:

The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

List of projects:

1. The production of chromosome aberrations in human lymphocytes by low doses of X-rays.

Title of the project no.: 1

The production of chromosome aberrations in human lymphocytes by low doses of X-rays

Head(s) of project:

Dr. D.C. Lloyd

Scientific staff:

Mr. A.A. Edwards
Mr. P. Finnon

I. Objectives of the project:

To irradiate blood in vitro to low doses of x-rays and to examine the lymphocytes in metaphase for radiation induced chromosome aberrations. The primary objective is to verify the existence of any low dose plateau in response over the range zero to a few tens of milligrays. Blood from 20 donors will be used because variations in sensitivity of donors may influence the low dose response. All cells containing exchange type aberrations will be photographed and karyotyped in order to determine whether certain chromosomes are specifically involved in such aberrations.

II. Objectives for the reporting period:

1. To complete scoring the material at 0 and 30 mGy
2. To decode and collate the results from the 6 collaborating laboratories
3. To analyse the data for:

   a) interlaboratory variation
   b) comparison of yields at 0 and 30 mGy
   c) possible donor variability
   d) multiply damaged cells
   e) karyotyping cells with exchange aberrations

III. Progress achieved:

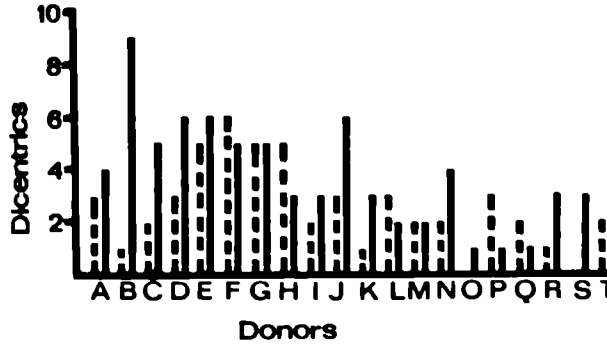
Methodology

Standard difference tests were applied to the data to determine the objectives a-c in section 2.

Results

Lab.	Dose (mGy)	No. Cells scored	Dicentrics	Centric Rings	Excess Acentrics
1	0	10500	14	1	63
	30	10200	3	3	66
2	0	10000	9	0	2
	30	10000	11	1	8
3	0	10015	10	0	28
	30	9980	23	2	39
4	0	10000	5	0	16
	30	10000	11	1	17
5	0	9797	8	0	16
	30	9718	16	0	34
6	0	10000	5	0	9
	30	10000	10	0	26
Total	0	60312	51	1	134
	30	59898	74	7	190

The pooled donor results from the 6 laboratories



The pooled laboratory results for dicentric aberrations in the 20 donors. For each donor 3,000 cells were scored for zero dose (broken lines) and 3,000 cells for 30 mGy (solid lines).

Discussion

As is usually experienced in collaborative projects some inter-laboratory differences in results are apparent. This is especially evident for the acentric aberrations reported by laboratories 1 and 2. We have found no particular explanation for this divergence. The total numbers of dicentrics scored by each laboratory are reasonably consistent but laboratory 1 is exceptional in having found significantly more dicentrics in the controls than in the irradiated cells. This is mainly due to the very low yield at 30 mGy but the zero dose yield is a little high. The latter includes one cell containing 3 dicentrics from donor F (see later).

For acentric aberrations the inter-laboratory variations tend to cancel out and the total results are approximately consistent with a linear extrapolation of published dose response data. For dicentrics the background yield is consistent with previous reports of approximately 1 per 1000 cells. The yield in the irradiated cells however is low when compared with the data reported by this group of collaborating laboratories in a previous experiment (see section 6) and by extrapolating other published higher dose data assuming the linear quadratic model. Even if one excludes the odd result from laboratory 1 the yield is still low but tests show that the significance is marginal. These data therefore tend to support the idea of a threshold or plateau effect in the very low dose response.

A test on the distributions of the control and exposed sets of dicentric data shown in the Figure indicated no significant deviation from the Poisson distribution (zero dose $\sigma^2/y = 1.03 \pm 0.3$, 30 mGy $\sigma^2/y = 1.13 \pm 0.3$). This suggests that if inhomogeneity exists within this group of 20 subjects it could only be detected by many more cells being scored. There is a suggestion from the data in the Figure that subjects with a low control yield also exhibited a low irradiation yield and conversely a high control is associated with a high irradiation yield. However this trend is not quite significant but will be subject to further testing when data from two more doses become available.

Three multiply damaged (>1 dicentric) control cells were observed; one in each of donors F, H and J, and one irradiated cell in donor N. This is a similar finding to the previous experiment (section 6) and is again significantly greater than expected from Poisson statistics.

Karyotyping the cells containing exchange aberrations is still in progress but no result is yet available.

Because of the anomalies and variations described above it was resolved to score the other cells irradiated at 5 and 300 mGy that were prepared at the same time. The slides have been distributed to the collaborating laboratories and scoring is in progress.

IV. Objectives for the next reporting period:

To complete scoring the the material irradiated at 5 and 300 mGy. To collate those results with the zero and 30 mGy data reported here. To analyse the full data in accordance with the objectives as described in sections 1 and 2 and to compare the results with those of a previous experiment now published (section 6).

V. Other research group(s) collaborating actively on this project (name(s) and address(es)):

1. State University of Leiden, Netherlands (Prof. A. Natarajan)
2. Free University of Berlin, Germany (Prof. G. Obe) (since transferred to University of Essen)
3. CEN/SCK Mol, Belgium (Dr. A. Leonard)
4. BNFL, Sellafield, UK (Dr. J. Tawn)
4. University of Rome, Italy (Dr. F. Palitti)

VI. Publications:

D.C. Lloyd, A.A. Edwards, A. Leonard, G.L. Deknudt, A. Natarajan, G. Obe, F. Palitti, C. Tanzarella, E.J. Tawn. Frequencies of Chromosomal Aberrations Induced in Human Blood Lymphocytes by Low Doses of X-Rays. Int. J. Radiat. Biol. 53 (1) 49-55, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-E-173-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. C. Tease
Genetics Div., Radiobiology Unit
Medical Research Council
Harwell, Didcot
GB - Oxon OX11 ORD

Telephone number: 0235-834393

Title of the research contract:

Karyotypic analyses of spontaneous and radiation-induced chromosome anomalies in mouse foetuses.

List of projects:

1. Karyotypic analyses of spontaneous and radiation-induced chromosome anomalies in mouse foetuses.

Title of the project no.: 1

Karyotypic analyses of spontaneous and radiation-induced chromosome anomalies in mouse foetuses

Head(s) of project: Dr. C. Tease

Scientific staff: Dr. C. Tease

I. Objectives of the project:

Mouse embryos with numerical chromosome anomalies, whether spontaneous or radiation-induced, are being investigated (a) to provide further information on the possible mechanisms of meiotic chromosome non-disjunction, and (b) to examine the particular involvement of different chromosomes in nondisjunction.

II. Objectives for the reporting period:

To compare the incidences of radiation-induced numerical and structural chromosome anomalies at different stages of embryo development.

III. Progress achieved:

Methodology

Female mice of the F_1 hybrid strain C3H/HeH x 101/H were induced to ovulate using 5 i.u. of PMS followed 48 h later by 5 i.u. of HCG. Three hours after HCG they were given 1 Gy of X-rays. One group of females was used for metaphase II oocyte preparations; a second group was mated and fertilised eggs used for one-cell embryo preparations. Both cell types were screened for numerical and structural chromosome aberrations.

Results

1. Metaphase II oocytes

Various types of chromosome anomaly were observed at metaphase II: the presence of an additional chromosome (hyperhaploidy); separation of the chromatids of one or more chromosomes (premature chromatid separation); and, structural anomalies, such as acentric fragments and dicentric chromatids. A low incidence of chromosome anomalies was observed in oocytes from untreated, control females (Table 1). After X-irradiation, the frequencies of all types of chromosome anomaly increased significantly compared with controls (Table 1). The largest increase occurred in the rate of structural chromosome anomalies.

Table 1. The proportions (%) of metaphase II oocytes from control and X-irradiated female mice with hyperhaploidy (n+1), premature chromatid separation (PCS) or structural chromosome anomalies (SA)

Treatment group	Number of females	Total cells analyzed	%		
			n+1	PCS	SA
Control	22	243	0.4	0.8	0.4
1 Gy	22	339	3.5	6.2	60.8

2. One-cell embryos

Chromosome anomalies in maternally-derived pronuclei were classified as hyperhaploidy (n+1) or structural (generally the presence of small, marker chromosomes). The incidences of numerical and structural chromosome anomalies were larger in embryos from irradiated females compared with untreated controls (Table 2). The most marked effect was for structural anomalies.

Table 2. The proportions (%) of one-cell embryos from control and X-irradiated female mice with maternally-derived hyperhaploidy (n+1) or structural chromosome anomalies (SA)

Treatment group	Number of females	Total cells analyzed	%	
			n+1	SA
Control	32	235	2.1	2.1
1 Gy	32	242	5.3	30.5

Discussion

The treatment of immediately preovulatory oocytes with 1 Gy of X-rays increased the incidences of aneuploid metaphase II oocytes and one-cell embryos. Comparison of the rates of aneuploidy in these two cell types is, however, hampered by (1) the large variation in spontaneous levels of nondisjunction between control groups. In particular, the frequency of hyperhaploidy in the one-cell embryos was larger than recorded previously using this technique; the reason for this is uncertain. The variability between untreated controls makes it difficult to assess the different rates of aneuploidy after X-irradiation. (2) The fate of prematurely separated chromatids in metaphase II oocytes is uncertain; it is not known whether or not they can contribute to embryonic aneuploidy through malsegregation at the second meiotic division.

The X-ray treatment given here induced an increase in aneuploidy in the two cell stages examined. In contrast, analysis of post-implantation embryos from females similarly treated has so far failed to detect a radiation-induced increase in aneuploidy. One possible explanation for these apparently discrepant outcomes, is that embryos with radiation-induced aneuploidies tend to die during pre-implantation development, perhaps due to the presence of induced genetic damage in addition to the aneuploidy.

IV. Objectives for the next reporting period:

To complete sampling and analysis of pre- and post-implantation embryos from control and irradiated females.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None

VI. Publications:

Tease C. Radiation-induced aneuploidy in mammalian germ cells. In "Somatic and Genetic Effects of Ionizing Radiation. XVth Berzelius Symposium" (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

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Contract no.: BI6-E-144-UK

Head(s) of research team(s) [name(s) and address(es)]:

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Title of the research contract:

DNA repair genes and the molecular basis of mutation and recombination in mammalian cells.

List of projects:

1. The molecular basis of mutation and recombination in mammalian cells differing in capacity to repair radiation damage.
2. The isolation and cellular and molecular characterisation of repair-deficient mammalian cell lines.
3. The cloning and analysis of radiation repair genes from lower organisms and their introduction into mammalian cells.

Title of the project no.: No 1. The molecular basis of mutation and recombination in mammalian cells differing in capacity to repair radiation damage.

Head(s) of project: Dr. J. Thacker

Scientific staff: Dr. J. Thacker

I. Objectives of the project:

To devise and implement methods for the molecular analysis of mutation and recombination of DNA in cultured mammalian cells of different repair capacity. Using recombinant DNA techniques the target molecules can be analysed for molecular changes and so reveal the nature of the events leading to mutation or recombination.

II. Objectives for the reporting period:

i) To measure induced mutant frequencies in the radiosensitive irs lines.

ii) Mapping and analysis of spontaneous and radiation-induced molecular deletions in the mammalian hprt gene.

III. Progress achieved:

1 METHODOLOGY

i) Mutation of the hprt gene was measured using our previously-published techniques, including the establishment of cultures carrying low spontaneous frequencies and auto-radiographic analysis of mutant clones.

ii) DNA was isolated from mutants which we had previously shown to carry partial deletions of the hprt gene - this was cut with various enzymes and analysed on Southern blots by hybridization to a full-length hamster hprt cDNA.

2 RESULTS

i) Experiments with a clone of the irs 3 line, selected for good cloning efficiency, showed that X-ray mutability (mutants per rad) was comparable to that of the V79 parent line. Similar experiments with irs 1 have been made difficult by a higher-than-normal spontaneous mutant frequency, which tends to increase with culture age. Selection of a clone of irs 1 with improved growth characteristics revealed an increased sensitivity to both the lethal and the mutagenic effects of X-rays, compared to the parental V79 cell line.

ii) DNA from 17 mutants (10 γ-ray induced, 5 α-particle induced, 2 spontaneous) was analysed in detail to locate the breakpoints of partial deletions in the hprt gene. This study was part of a collaboration with workers at Pacific Northwest Laboratories who have a similar number of partial deletion mutants. DNA fragment patterns with 3-4 enzymes were compared to a partial map of the hamster hprt gene (kindly supplied by Drs. B. Rossiter & M. Fox, Paterson Institute, Manchester). Breakpoints were found in all parts of the gene, although there was some clustering in the region of exons 4 and 5.

3 DISCUSSION

i) We showed previously that the irs 2 line was not altered in mutability when compared to the parent V79 cells; now we have shown this true also for irs 3. This result further emphasises that the endpoints of cell killing and mutation can be uncoupled, ie, some radiation-induced damage is lethal but does not lead to mutation. On this basis these radiosensitive lines have similarities to cells from the human disorder ataxia-telangiectasia (see Project 2 also). The irs 1 mutant is clearly different, with an elevated X-ray mutability, although the relationship between cell survival and induced mutant frequency following X-irradiation is altered little when compared to that of the parental cell line.

ii) The location of breakpoints is a necessary first step to their molecular analysis. In addition, the finding of some clustering of breakpoints may indicate specific sequences in the hprt gene are more prone to involvement in radiation-induced deletion formation than the remainder of the gene. This analysis of breakpoints at the DNA sequence level should, in this way, lead to a better understanding of mechanisms of radiation action.

IV. Objectives for the next reporting period:

To analyse the size of molecular deletions already identified in hamster hprt mutants, using pulse-field gel electrophoresis. To isolate spontaneous and X-ray induced hprt gene mutations in human cells and to identify those with partial and complete gene deletions. These mutants will ultimately be used for detailed analysis of deletion breakpoints.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

DEBENHAM, P.G., M.B.T. WEBB, A. STRETCH and J. THACKER
Examination of vectors with two dominant selectable genes for DNA repair and mutation studies in mammalian cells. Mutation Research, 199, (1988) 145-158.

THACKER, J. and A. GANESH
Molecular analysis of spontaneous and ethyl methane sulphonate-induced mutations of the hprt gene in hamster cells. Mutation Research, in press.

Title of the project no.: 2. The isolation and cellular and molecular characterization of repair-deficient mammalian cell lines.

Head(s) of project: Dr. J. Thacker

Scientific staff: Dr. J. Thacker, Dr. T.C. Brown (from May 1988)

I. Objectives of the project: To understand the repair processes acting on ionising radiation damage in mammalian cells, through the analysis of mutants defective in different repair functions. In particular we will apply both cellular and recombinant DNA techniques to the characterisation of newly-isolated mutants sensitive to ionising radiation. Ultimately these studies should identify both the important type(s) of DNA damage caused by ionising radiation and the nature of the repair enzymes acting on this damage.

II. Objectives for the reporting period:

i) To assess the number of genes affecting radiation sensitivity by forming hybrids between radiosensitive mutants isolated in different laboratories and testing their survival (and other responses as required).
ii) To analyse the rejoining of DNA double-strand breaks and resistance of DNA synthesis to radiation damage in the radiosensitive irs lines.
iii) To develop a method of examining the repair of DNA damage in vitro using human cell-free extracts (especially from ataxia-telangiectasia patients).
iv) To identify and isolate repair proteins by their association with viral DNA molecules irradiated in mammalian cells.

III. Progress achieved:

1. METHODOLOGY

i) Fusion of genetically-marked radiosensitive lines in pairs using polyethlene glycol, followed by selection of hybrids in appropriate media.

ii) a) Alkaline or neutral sucrose gradient sedimentation of DNA from cells after labelling with ^{3}H-thymidine and γ-irradiation (10 or 20 krad).

b) Double-labelling of cells, firstly with ^{14}C-thymidine to measure overall synthetic capacity, followed by irradiation (on ice) and then measurement of postirradiation synthesis by ^{3}H-thymidine incorporation. The time course and dose-dependence of DNA synthesis was measured for each mutant (or hybrid; see below).

iii) A suitable plasmid carrying a multiple-enzyme break site in the lacZ gene was chosen to act as substrate in which various types of DNA termini could be produced (initially we have worked mainly with cohesive-ended breaks but latterly with blunt-ended breaks at the site). These broken molecules were treated with extracts from human cells under defined conditions and rejoining was assessed by gel electrophoresis. Additionally treated DNA molecules were used to transform suitable bacteria and the fidelity of rejoining assessed by activity of the lacZ gene.

iv) CV-1 cells were infected with SV40 virus and irradiated with UV light or γ-rays. The SV40 'minichromosomes' were isolated from cell nuclei and fractionated on sucrose gradients. Proteins associated with the mini-chromosomes were analysed on SDS (Laemmli) gels.

2. RESULTS

i) Fusion hybrids between cultures from different patients with the radiosensitive disorder ataxia-telangiectasia (transformed lines of AT4BI and AT5BI) were found to have AT survival characteristics (non-complementing; ie, involving the same gene). This result was contrary to previous reports which used single-cell hybrids and the radioresistance of DNA synthesis as the measured endpoint. We therefore measured this endpoint on our bulk-culture hybrids, but again found an AT-like radioresistance (compared to hybrids of these AT lines with normal human cells).

We have also continued our complementation study of the available X-ray sensitive rodent cell lines without finding any further lines which derive from the same complementation group (extensive hybridizations with mutants irs 1, irs 2, irs 3, xrs-1, EM7, XR-1, BLM2 and more limited hybridizations with V-E5, V-G8, irs 1SF and M10).

ii) Rejoining of both single- and double-strand breaks, in time-course experiments using sucrose gradient sedimentation, was similar for all the irs mutants and the parental V79 cell line. However, the radioresistance of DNA synthesis varied in the different irs mutants. irs 2 showed the largest resistance, although the difference between it and V79 diminished with dose. irs 1 also showed some resistance but irs 3 was similar to V79. Time-course experiments showed that, even for irs 2, the relative radioresistance of DNA synthesis in these rodent mutants was not as great as that found for AT human cells.

iii) Broken DNA was visualised on gels and led to a 500-fold reduction in transformation frequency of bacteria. Treatments of this DNA with cell extracts initially yielded only multimers of linear molecules but refinement of reaction conditions led to the formation of circular monomers. No rejoining was seen with boiled control extracts.

Transformation of bacteria was increased considerably by extract treatment compared to untreated or boiled extract-treated DNA, and the proportion of transformants with inactive lac gene function was very low. This suggests that the rejoining of broken (cohesive-ended) molecules is relatively error-free. In contrast, control experiments (eg, infilling the cohesive ends using DNA polymerase, then ligating the molecules) gave a high proportion of transformants with an inactive lac gene, illustrating the detection properties of the system. At present we are investigating the rejoining of blunt-ended breaks, with the use of cell extracts from both normal and AT human cells.

iv) SV40 minichromosomes were isolated from unirradiated and UV-irradiated cells and their associated proteins analysed on gels. About 100 protein bands were visible, some of which were ribosomal proteins. The UV treatment provoked 3 or 4 distinct changes in the protein profile, and in particular two proteins of 220 and 230 kd were identified. Such changes in protein profile have not been detected so far with γ-irradiation of the virus.

3. DISCUSSION

i) The non-complementation of two ataxia-telangiectasia (AT) lines suggests that the genetic complexity of this disorder may be less than previously thought. However, our work with various radiosensitive rodent mutants shows that at least 9 or 10 different complementation groups (= genes) determine resistance to radiation damage. Since only one of these complementation groups has been found to contain mutants isolated in more than one laboratory, it is likely that there are many more groups influencing radiosensitivity to be found.

ii) All of the irs mutants are shown to be like human AT cells in that they have approximately 3-fold increased radiosensitivity and no obvious reduction in DNA single- and double-strand break rejoining. However, only irs 2 is similar to AT in having a marked resistance to radiation-induced DNA synthesis delay. In other respects (see previous reports) AT cells were more like irs 1 cells, although unlike AT this mutant has high sensitivity to mitomycin C. Thus these different mutants show overlapping phenotypic characteristics when compared to the major human radiosensitive disorder.

iii) We have demonstrated for the first time the ability of human cell extracts to rejoin broken recombinant DNA molecules. This is the initial step in an examination of the ability of extracts to repair various types of damage, and suggests that at least some DNA metabolizing enzymes are active in our in vitro conditions.

iv) The use of a virus which replicates after infection to give a large number of copies in the cell has allowed the analysis of proteins associated with irradiated chromatin. It remains to be demonstrated that such proteins are active in the repair of damaged chromatin.

IV. Objectives for the next reporting period:

To complete cell complementation studies for the various rodent (especially mouse x hamster) radiosensitive mutants. To assess the low-dose-rate response of the irs hamster mutants, compared to the parent V79 cells (lethal damage recovery potential). To examine the ability of human cell extracts, especially from AT cultures, to repair various types of breaks in recombinant DNA molecules and to assess the fidelity of that repair.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

JONES, N.J., R. COX and J. THACKER
Six complementation groups for ionising-radiation sensitivity in Chinese hamster cells. Mutation Research, 193, (1988) 139-144.

HERSKIND, C. and J. THACKER
Inactivation of DNA-mediated transformation of hamster cells by γ-rays and deoxyribonuclease I. Mutation Research, 198, (1988) 169-178.

THACKER, J. and P.G. DEBENHAM
The molecular basis of radiosensitivity in the human disorder ataxia-telangiectasia. In Mechanisms and Consequences of DNA Damage Processing, UCLA Symposia on Molecular and Cellular Biology, New Series (Eds E. Friedberg and P. Hanawalt). A.R. Liss: New York, Vol.83, 1988, pp 361-369.

DEBENHAM, P.G., M.B.T. WEBB, A. STRETCH and J. THACKER
Examination of vectors with two dominant selectable genes for DNA repair and mutation studies in mammalian cells. Mutation Research, 199, (1988) 145-158.

THACKER, J
The use of integrating DNA vectors to analyse the molecular defects in ionising radiation-sensitive mutants of mammalian cells including ataxia-telangiectasia. Mutation Research, in press.

THACKER, J., R. WILKINSON, A. GANESH and P. NORTH
Mechanisms of resistance to ionising radiations: genetic and molecular studies on ataxia-telangiectasia and related radiation-sensitive mutants. In DNA Repair Mechanisms and their Biological Implications in Mammalian Cells, (Eds: M.W. Lambert et al) Plenum Press: New York. In press.

Title of the project no.: No. 3. The cloning and analysis of radiation repair genes from lower organisms and their introduction into mammalian cells.

Head(s) of project: Dr. J. Thacker

Scientific staff: Dr. F.E. Benson and Dr. J. Thacker

I. Objectives of the project:

Much is known of the mechanisms of DNA repair in microbes; many repair deficient mutants have been isolated, many repair genes cloned and many proteins involved in repair have been identified. In contrast, little is known of equivalent processes in mammalian cells. To capitalise on the wealth of repair data from microbes it is proposed to transfer cloned and characterised microbial repair genes into radiosensitive and normal mammalian cells. In this manner it is planned to assess genetic complementation or perturbation of endogenous repair pathways so as to functionally characterise repair processes/defects in mammalian cells.

II. Objectives for the reporting period:

To completely sequence the *rorB* gene, identify the reading frame, and compare the gene to other sequenced repair genes (and other genes of interest in available sequence data banks). To assess sequence homologies between the *rorB* gene and genes in other organisms using Southern blot techniques. In addition to the above objectives, a study of the possible interactions between the *rorA*, *recB* and *recD* genes was initiated.

III. Progress achieved:

1 METHODOLOGY/RESULTS

a) The nucleotide sequence of a 1.5 kb PstI to EcoRI restriction fragment, known to contain the rorB gene, has been determined. Overlapping fragments of approximately 200 bp each were subcloned into the M13 vectors mp18 and mp19, and their nucleotide sequence determined by the dideoxynucleotide chain termination method. Computer analysis of the nucleotide sequence revealed the presence of an open reading frame (ORF1), that could code for the RorB protein. This reading frame is preceded by a sequence with homology to the E.coli consensus promoter sequence, and the proposed GTG initiation codon preceded by a possible ribosome binding site. Translation of this reading frame would result in a polypeptide of approximately 25 kd, which is somewhat larger than that previously identified in maxicell extracts of strains carrying rorB+ plasmids.

The nucleotide sequence and the amino acid sequence of the predicted protein have been used to search the GenBank and PIR databases respectively. A search of the nucleotide sequence database revealed that approximately 300 bp downstream of the termination codon for ORF1 were two tRNA genes, tRNAasp and tRNAtrp, at the 3' end of the rrnC operon. No other significant nucleotide sequence homologies were detected. A search of the protein sequence database revealed that the predicted product of ORF1 was similar to the LysR group of bacterial activator proteins, and in particular to the IlvY product, which activates transcription of the IlvC gene.

In order to confirm that the identified reading frame corresponds to the rorB gene, a series of plasmid deletion derivatives have been constructed, harbouring various restriction fragments derived from the rorB region.

b) The proposed study of sequence homologies between the rorB gene and similar genes in different organisms has not been initiated, since it was concluded that the precise coding region of the rorB gene had to be defined before suitable probes could be chosen for such a study.

c) The rorA mutation, which results in an increased sensitivity to gamma irradiation, whilst having no effect on UV irradiation sensitivity, has been transferred into a well characterised genetic background, and the phenotype it confers confirmed. The ability of plasmids carrying the recB, recC and recD genes to complement the gamma irradiation sensitivity of rorA strains has also been investigated. However, although we have eliminated the possibility that the rorA mutation is an allele of recC, we have not yet reached a conclusion as to whether rorA is an allele of recB or recD. This is in part because we have found that multicopy plasmids carrying the recD+ gene have a negative effect on the survival after gamma irradiation of wild type cells.

2 DISCUSSION

The above studies suggest that the *rorB* gene encodes a protein similar to the LysR group of bacterial activator proteins, and in particular to the IlvY protein. Since the *rorB* gene is situated approximately 1 kb upstream of the *ilvGEDA* operon it could be postulated that the RorB protein may have some role in the regulation of this operon. Evidence to support this comes from previous studies that demonstrate that a protein encoded by the same region of DNA to which we have localised the *rorB* gene may be regulated along with the *ilvG* gene. If the main role of the RorB protein is regulation of this operon, then it is at first difficult to reconcile such a role with the observation that *rorB* mutations result in an increased sensitivity to gamma irradiation. However, it may be that RorB protein, like integration host factor (which also affects regulation of the *ilvGEDA* operon) has many roles in cellular metabolism, arising from its ability to bind DNA.

IV. Objectives for the next reporting period:

a) To investigate the ability of plasmids carrying fragments derived from the *rorB* region to restore the radiation resistance to *rorB* mutant strains, and thus confirm that the identified reading frame does in fact code for the RorB protein. To analyse the proteins produced in maxicell extracts of strains harbouring the plasmid deletion derivatives that complement the *rorB* radiation sensitivity.
b) To assess sequence homologies between the *rorB* gene and genes in other organisms using Southern blot techniques.
c) To further investigate the relationship between the *rorA*, *recB* and *recD* genes.
d) To analyse *recA*-containing clones of normal and radiosensitive (A-T) cells, assessing the production of RecA protein and its effect on various cellular responses.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

None.

VI. Publications:

DEBENHAM, P.G. and WEBB, M.B.T. (1988)
The isolation and preliminary characterisation of a novel *Escherichia coli* mutant *rorB* with enhanced sensitivity to ionising radiation. *Molecular and General Genetics* 215: 161-164

DEBENHAM, P.G., WEBB, M.B.T. and LAW, J. (1988)
The cloning of the *rorB* gene of *Esherichia coli*. *Molecular and General Genetics* 215: 156-160.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-167-NL

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Head(s) of research team(s) [name(s) and address(es)]:

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Title of the research contract:

Processing of radiation induced and spontaneous genetic damage in prokaryotes and eukaryotes.

List of projects:

1. Processing of radiation induced and spontaneous genetic damage in prokaryotes and eukaryotes.

Title of the project no.: Processing of radiation induced and spontaneous genetic damage in prokaryotes and eukaryotes. (Mechanisms of DNA repair in mammalian cells).

Head(s) of project: Prof.Dr. P. van de Putte
Dr. C. Backendorf

Scientific staff: Drs. P. Belt
Drs. S. Gibbs
Mrs. W. Teubel

I. Objectives of the project:

- Study of the influence of UV irradiation on cultured human keratinocytes, Identification of UV inducible genes. Analysis of the induction process.
- Characterization of DNA repair systems in human cells. Cloning and characterization of the genes defective in xeroderma pigmentosum.

II. Objectives for the reporting period:

- Characterization of UV inducible genes. Isolation of genomic clones for spr1 and spr2. Characterization of the spr2 promoter.
- Cloning of the XP-A gene. Isolation of UV resistant XP transfectants. Microinjection of *in vitro* synthesized RNA into primary XP cells.

III. Progress achieved:

1. UV inducible genes in human keratinocytes:

a) Methodology

SPR genes code for small proline rich proteins and have been isolated from a human keratinocyte cDNA library as UV and TPA inducible genes. During the last contract period a human genomic library constructed in the lambda vector EMBL3 was screened with cDNA probes from the spr1 and spr2 genes. In each case positive lambda clones were picked up and partially sequenced in order to determine the chromosomal structure of the spr1 and spr2 genes. A hybrid mouse-human cell panel was screened with specific probes in order to determine the chromosomal localization of the spr genes.

b) Results

- Isolation of the promoter of one member of the spr1 gene family: Screening of the lambda EMBL3 human genomic library resulted in the isolation of two different lambda clones which hybridize with the spr2 cDNA probe. λ-spr2-1 harbors two members and λ-spr2-2 one member of the spr2 gene family. One of the two spr2 genes on λ-spr2-1 was analysed in detail. The gene is composed of two exons interrupted by an intervening sequence of approximately 800 basepairs. The whole spr2 coding sequence is localized on exon2. Exon1 is only 42 bp long and could be localized on the lambda clone with the use of a 25 bp long synthetic oligo-nucleotide; it is preceded by the spr2 promoter. Until now 600 base-pairs of the spr2 promoter have been sequenced and the following features have been observed: An RNA polymerase binding site (TATA box) is found at position -25. At position -193 the consensus sequence for a TPA responsive element (TRE) is found. As we have shown (last year's report) that the spr2 gene is regulated by TPA it is very well possible that the sequences around position -193 are actually involved in this regulation. Deletion mapping and promoter fusion to the cat reporter gene are necessary to clarify this point. The spr2 promoter is characterized by a large number of repeated structures where the most prominent one is a duplication of 55 bp in the -300 region. As spr2 is specifically expressed in keratinocytes a number of these sequences might be involved in cell type specific expression. Furthermore at position -54 an interferon responsive element (IRE) has been detected. As interferons are potent inducers of terminal differentiation in cultured keratinocytes it will be interesting to analyse whether these sequences are implicated in the regulation of spr genes during terminal differentiation (see last years's report).

- Identification of a new member in the spr gene family: the lambda EMBL3 library was also screened with an spr1 cDNA probe in order to isolate the spr1 promoter. Although a lambda clone hybridizing to the spr1 probe could be isolated, DNA sequencing revealed that the isolated clone did not correspond to the spr1 gene but to a spr1 related new member of the spr gene family, denominated spr3. The spr1 and spr3 proteins have similar N- and C-terminal sequences but differ in the middle part of the proteins which is constituted of repetitive motives of an octapeptide. In spr1 the sequence PKVPEPC* is repeated 6 times whereas in spr3 the sequence TKVPEPGC is repeated 13 times. The dif-

ference in the middle part of both proteins made it possible to design specific probes for each of the two genes. Recent experiments with the spr3 specific probe revealed that the spr3 gene is indeed transcribed and that the spr3 mRNA is slightly longer than the spr1 transcript. It will be interesting to analyse whether the expression of both genes is similar after UV or TPA treatment or during terminal differentiation of cultured keratinocytes.

- Chromosomal location of spr genes: The spr genes constitute a multigene family where at least the spr2 gene is present in multiple copies in the human genome. In order to determine whether spr genes are localized on one or several human chromosomes a mouse/human hybrid cell line panel was screened with specific probes for spr1, spr2 and spr3 in collaboration with Dr. A. Geurts van Kessel at the University of Rotterdam. From the results obtained it is clear that all spr genes are localized in the central part of human chromosome No. 1. Fine mapping of the exact position of these genes on chromosome 1 is underway.

c) Discussion

Recent work, in a number of laboratories, has shown that unrepaired DNA damage does not only induce mutations in living cells but also a temporary stress response reminescent of the bacterial SOS system. Since several years the department of Molecular Genetics is involved in studying these phenomena in cultured human keratinocytes. A number of UV inducible genes have been identified among which the spr genes have been studied in more detail. The spr gene family is composed of 3 subfamilies of genes which we have denominated spr1, spr2 and spr3. The exact number of copies per haploid genome of each of these genes is still under investigation and not exactly known. The spr2 subfamily is present in the human genome at approximately 8-10 copies. Genomic clones from 3 of these spr2 genes have been isolated. From one gene the promoter was identified and sequenced; this will allow the study of the regulation of this gene on a molecular level (fusion to the cat reporter gene; deletion mapping). It will be interesting in the future to isolate promoter sequences from other members of the spr2 family in order to establish whether all spr2 genes are similarly regulated or whether different spr2 genes react differently to different stimuli. Furthermore the isolation of promoter sequences will enable us to study and identify the transactivating proteins involved in the regulation of these genes. In particular it will be important to establish whether the AP-1 system (oncogenes jun and fos) which has been shown to be involved in the regulation of TPA responsive genes in human fibroblasts (and HeLa cells) is also implicated in the regulation of spr genes in primary keratinocytes. The presence of a TRE (=AP-1 binding site) in the promoter region of spr2 suggests that this might be the case. In the last year's report we have shown that expression of the spr1 gene was not influenced by UV irradiation although it was induced after TPA treatment. These results, however, were obtained with a cDNA probe which cross-hybridizes with the newly identified spr3 gene. Consequently the expression of spr1 and spr3 both after UV or TPA treatment and during terminal differentiation have to be reinvestigated with specific probes for each of the two genes before starting to isolate promoter sequences specific for spr1 and spr3 genes. In order to localize the spr3 promoter full length cDNA clones have to be obtained first.

2. Cloning of the xeroderma pigmentosum group A gene

a) Methodology

During the last few years two cDNA expression libraries have been constructed from size fractionated poly A^+ RNA which contained the mRNA coding for the XP-A protein (as shown by microinjection of this RNA pool into XP-A cells and subsequent correction of the XP repair defect). During the last period experiments were performed in order to isolate an XP-A cDNA clone from these two libraries. The EBV expression library was transfected to SV40 transformed XP-A fibroblasts and UV resistant transfectants were selected. The T7 expression library was used to synthesize RNA in vitro. This RNA was subsequently capped and microinjected into primary XP-A fibroblasts followed by in situ repair synthesis measurements.

b) Results

EBV vector system: In 4 independent experiments with two different libraries (25.000 and 75.000 different cDNA inserts respectively) more than 100 dishes of XP_2OS-SV fibroblasts (10^6 cells/dish) were transfected and more than $5x10^4$ hygromycin resistant transformants were obtained which were subsequently challenged with UV light. In all these experiments one single UV resistant clone (denominated 6-4) was isolated from one dish after transfection with the "25.000" library. The use of a human polymorphic repetitive probe confirmed that the UV resistant clone was indeed derived from the UV sensitive XP_2OS-SV mother population. UV survival curves established that the 6-4 clone was about 70-80% resistant as compared to repair proficient fibro-blasts or HeLa cells. A similar conclusion was reached after UDS measurements. In order to rescue a possible XP-A correcting EBV vector from the 6-4 clone low molecular weight DNA extractions were performed and transformed to E. coli. However, no ampicilline resistant bacterial colonies could be obtained. The reason for this failure turned out to be due to the fact that in 6-4 clones no episomal copies of the EBV vector were present anymore but rather that one copy of the vector had integrated into the host genome. Moreover the cDNA expression vector had been fractionated into at least several parts which will make the rescue of a correcting cDNA insert extremely difficult. Indeed at present the only vector sequences we still can detect are part of the ampicilline gene, the hygromycin selection marker and part of the RSV promoter. Unfortunately a break occurred between the RSV promoter and the cDNA insert.

T7 expression system: RNA was synthesized in vitro from sublibraries harbouring either 600 or 6000 different clones. A large number of XP-A fibroblasts were injected, however no positive effect on repair synthesis could be detected although microinjection of the original natural RNA population used to construct the library was positive in this assay. In order to analyse whether in vitro synthesized RNA with T7 polymerase is as efficient in correcting a genetic defect after microinjection into human cells as natural RNA, a T7 expression vector harbouring the hgprt gene was used to synthesize RNA in vitro. Although highly concentrated RNA preparations were able to correct $hgprt^-$ human cell lines, an only 5-fold dilution of the RNA preparation already abolished the effect. These results strongly suggest that in vitro synthesized RNA is rapidly degraded in mammalian cells after microinjection and that the T7 approach is probably useless for screening complex libraries.

Discussion

In transfection experiments one single UV resistant clone was isolated from more than 100 transfected XP_2OS-SV dishes. This frequency is much lower than what we had expected from reconstruction experiments. Here if a single clone was present in a mixture of 20.000 it could be rescued almost on each dish. Supposing that the XP-A gene is present in the "25.000" library and that the 6-4 clone has been corrected by this cDNA insert than some extra factors must be involved in order to explain the low frequency with which UV resistant clones are obtained. in E. coli we have observed that overexpression of the uvrA repair gene can be deleterious for the cell. As the EBV vectors constitute a high expression system (50 copies/cell and a strong RSV promoter directing the expression of the cDNA insert) it is not impossible that the results obtained are due to a toxic effect caused by the overproduction of the XP-A protein. The findings that in the UV resistant 6-4 transfectant no episomal copies of the vector are present and that at least one break has occurred between the RSV promoter and the cDNA insert during the integration process are not in contradiction with such a view. Nevertheless with the data obtained one could as well argue that the 6-4 clone is a mere revertant of the XP-A phenotype. However, we feel that a number of observations argue against such an explanation: if the 6-4 clone is a revertant than there is no obvious reason why the episomal vector (50 copiers/cell originally!) is finally present as one single integrated copy. Since the isolation of the 6-4 clone we have analysed a large number of human cell clones transfected with the EBV library and subsequently irradiated many times with UV but in no case we have found a similar feature. Indeed all clones analysed still harboured episomal vectors. Integration of EBV vectors does apparently not occur with high frequency. As reversion of the XP-A phenotype can only occur at very low frequencies (in the order of 10^{-7} in XP_2OS) we believe that the probability that integration and reversion occur in one clone is very low.

IV Objectives for the next reporting period

1. UV regulated genes in human keratinocytes:

- Indentification of the sequences involved in the regulation of the spr2 gene family. Deletion mapping. CAT assay.
- Further characterization of the spr gene family and the regulated expression of the different spr genes.

2. Cloning of the XP-A gene:

- Further analysis of the 6-4 clone in order to isolate the correcting cDNA clone. Use of synthetic oligonucleotides in order to detect integrated cDNA copies
- More transfections, as well to cells from other XP-A patients, in order to obtain more UV resistant transfectants.

V Other research group(s) collaborating actively on this project [name(s) and address(es)]

- Dept. of Genetics and Cell Biology (Prof. D. Bootsma) Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, NL.
- Leiden University Hospital, Dept. of Dermatology (Dr. M. Ponec) Rijnsburgerweg 10, 2333 AA Leiden, Nl.

VI Publications

- Kartasova, T., Ponec, M. and Van de Putte, P. (1988) Induction of proteins and mRNAs after UV irradiation of human epidermal keratinocytes. Exp. Cell. Res. 174, 421-432.
- Kartasova, T. and Van de Putte, P. (1988) Isolation, characterization and UV-stimulated expression of two families of genes encoding polypeptides of related structure in human epidermal keratinocytes. Mol. Cell. Biol. 8, 2195-2203.
- Kartasova, T., Van Muijen, G.N.P., Van Pelt-Heerschap, H. and Van de Putte, P. (1988) A novel protein in human epidermal keratinocytes. Regulation of its expression during differentiation. Mol. Cell. Biol. 8, 2204-2210.
- Belt, P.B.G.M., Groeneveld, H., Teubel, W.J., Van de Putte, P. and Backendorf, C. (1989) Construction and properties of an EBV-derived cDNA expression vector for human cells. Submitted to Gene

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-169-NL

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. A.J. van der Eb
Department of Medical Biochemistry
Sylvius Laboratoria
P.O. Box 9503
NL - 2300 RA Leiden

Telephone number: 071-276115

Title of the research contract:

The genetic and biochemical basis of radiation sensitivity in human and other mammalian cells in culture.

List of projects:

1. DNA repair and mutagenesis.

Title of the project no.:
DNA repair and mutagenesis
Contract no. BI6-E169-NL

Head(s) of project:

Prof.Dr. A.J. van der Eb
Department of Medical Biochemistry
Sylvius Laboratoria P.O. Box 9503
2300 RA Leiden, The Netherlands

Scientific staff:

Dr.P.J. Abrahams
Vacancy

I. Objectives of the project:

Exposure of bacteria to various DNA damaging agents results in the transient activation of a number of phenomena, called SOS-functions. The purpose of this project is to investigate whether SOS-like responses can be induced in mammalian cells by treatment with DNA damaging agents. Our working hypothesis is that such phemomena might be responsible for genetic alterations in cells that can initiate certain steps in carcinogenesis. Our studies are concentrating on the possible relationships between SOS-functions, mutations and cancer and the identification of processes involved in the SOS-response.

II. Objectives for the reporting period:

Previous work in our laboratory has shown that in normal human cells and some Xeroderma pigmentosum (XP) cells, certain SOS-like phenomena, such as Enhanced Reactivation (ER) and Enhanced Mutagenesis (EM) are induced after UV-treatment of the cells. However, in some of the XP cells studied only induction of the EM phenomenon was observed, whereas the ER response was absent. Interestingly, the absence of ER (ER^-) could be correlated with the absence of tumors in the patients on sunlight-exposed skin aereas, whereas XP cells exhibiting the usual ER response (ER^+) were derived from patients that were cancer prone. These results suggested that the ER response could somehow be involved in the process of oncogenic transformation.

The objective of this project in 1988 was to study the induction of the ER response in various heriditary cancer-prone syndromes, in order to obtain more information on a possible correlation between the ER phenomenon and carcinogenesis. In addition, the ER^+ and the ER^- XP cells were also characterized in other properties, such as the stabilization of the p53 cellular tumor antigen after UV-treatment.

III. Progress achieved:

1. Methodology

Cultures of normal human diploid skin fibroblasts and skin fibroblasts from the following cancer-prone syndromes: Wilm's tumour, Retinoblastoma, Polyposis coli, von Recklingshausen's neurofibromatosis, Displastic naevus syndrome and Bloom's syndrome, were exposed to various UV-doses in order to induce SOS-like functions. Cultures were infected with unirradiated or UV-irradiated Herpes simplex virus (HSV-1) 24 hours after UV-treatment of the cells in order to quantitate the ER-response. The stabilization of the cellular p53 tumor antigen was studied after UV-treatment of normal human skin fibroblast and of ER^+ and ER^- cells. Shortly after UV-exposure, cultures were labeled with ^{35}S-methionine for a few hours, after which they were chased with non-radioactive medium for various time intervals. Using an anti-p53 monoclonal antibody, the p53 cellular tumor antigen was specifically immunoprecipiated and the protein complexes were analyzed by SDS-PAGE.

2. Results

We have previously shown that ER and EM are maximally expressed 24-48 hrs after UV-treatment of normal human skin fibroblasts and some XP cells ($XP\text{-}ER^-$). It was noticed that the $XP\text{-}ER^+$ cells were derived from XP patients exhibiting skin cancer as usual, whereas the $XP\text{-}ER^-$ cells were derived from patients that were reportedly free of skin tumors at the time they were described in the literature. This observation suggested that the ER response may possibly be related, directly or indirectly, to the proces of carcinogenesis. In order to investigate this phenomenon further, we have determined the dose-dependence of the ER response in the following hereditary cancerprone syndromes: Wilm's tumour (WT), Retinoblastoma (Rb), Polyposis coli (PC), von Recklingshausen's neurofibromatosis (Rd), Dysplastic Naevus syndrome (DNS) and Bloom's syndrome (BS). Interestingly, we observed in all these syndromes much higher levels of ER than in normal human skin fibroblasts. For example, at a UV-dose of 20 $J.m^{-2}$ an ER of 2.4, is found in normal cells whereas an ER of 10 was observed in WT, PC and DNS. These results support our previous observation that the ER response might somehow be involved in the process of carcinogenesis, although the appearance of a similar phenomenon in genetically different hereditary diseases is difficult to explain. An unexpected result was obtained with cells from Wilm's tumour patients. We observed that the survival of UV-treated HSV was much lower in skin fibroblasts from 3 out of 4 WT patients, compared to the survival observed in normal human skin fibroblasts, suggesting that cells from these WT patients might have a DNA repair deficiency.

Recently it has been reported that skin fibroblasts from Trichothiodystrophy patients (TTD) are hypersensitive to UV-light. We also found in TTD cells a much lower survival of HSV than in normal human cells: Survival at a UV-dose of 40 $J.m^{-2}$ to HSV in TTD is 2.5×10^{-3}, in XP-D 2.3×10^{-3} and in normal cells 1.8×10^{-1}.

In order to characterize the $XP\text{-}ER^+$ and $XP\text{-}ER^-$ cells further we have also studied the UV-induced stabilization of the p53 cellular tumor antigen. In normal human cells a considerable stabilization of the p53 protein is observed after UV-exposure in pulse-chase experiments. The half-life of p53 in UV-exposed cells is about 7 hours, whereas in untreated cells the half-life is 2 hours. Similar pulse-chase experiments were carried out with $XP\text{-}ER^+$ and $XP\text{-}ER^-$ cells. It was found that in all

the XP cells tested so far (with one exception) little if any stabilization of the p53 protein was observed. However, no correlation was observed between expression of the ER response and stabilization of p53 protein in XP-cells. To investigate whether UV-treatment of normal human cells might cause overall stabilization of cellular proteins, pulse-chase experiments were carried out with whole lysates. No evidence for overall-stabilization of proteins was found. Therefore, our experiments suggest that UV-treatment of normal human cells results in stabilization of p53 and possibly of some other specific cellular protein(s), whereas this does not take place in repair deficient XP cells.

3. Discussion

Our results have shown that the ER^- response can be induced to very high levels in various cancer prone syndromes but not in normal skin fibroblasts. ER induction is also aboserved in XP cells of various complementation groups, but ER is apparently absent in XP cells from patients which do not show the normal pattern of cancer induction in sunlight-exposed areas of the skin. These results show that a correlation exists between ER and cancer induction. This is also suggested by the observation that an unusually high ER response is found in various cancer-prone genetic diseases. Why these genetically different diseases all show an abnormal ER response is unclear. Unexpectedly, some of the WT cells exhibited UV-sensitivety suggesting a deficiency in the DNA-repair mechanism. We are now investigating which step in the DNA-repair mechanisms of these WT cells is defective. In addition, we are presently studying expression of various UV- or TPA-inducible genes in the $XP\text{-}ER^+$ and $XP\text{-}ER^-$ cells by Northern-blot hybridization or immunoprecipitation. So far we have found that the p53 oncogene product is stabilized transiently after UV-irradiation but that stabilization apparently does not occur in XP cells.

IV. Objectives for the next reporting period:

see paragraph 3

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs. G. Hilgers, J.J. Cornelis, J. Rommelaere, Laboratoire de Biophysique et Radiobiologie, Université Libre de Bruxelles, Rue de Chevaux 67, 1640 Rhode St. Genèse, Belgium.

Prof. P. van der Putte, Department of Molecular Genetics, Wassenaarseweg 64, University of Leiden, The Netherlands.

Prof. P. Herrlich, Krebsforschungszentrum Karlsruhe, Institut für Genetik and für Toxibiologie and Institut für Genetik der Universität Karlsruhe, Postfach 3640, D-7500 Karlsruhe, F.R.G.

VI. Publications:

P.J. Abrahams, B.A. Huitema and A.J. van der Eb. Enhanced Reactivation and Enhanced Mutagenesis of Herpes simplex virus in normal human and Xeroderma pigmentosum cells. Mol.Cell.Biol. 11(1984)2341-2346.

E.H.A.Pol, P.J. Abrahams, F. Arwert and A.W. Erikson. Host cell reactivation of cis-deaminedichloroplatinum(II)-treated SV40 DNA in normal human, Fanconi anemia and Xeroderma pigmentosum fibroblasts. Mut. Res. 132(1984)181-187.

C.Dinsart, J.J. Cornelis, B. Klein, A.J. van der Eb and J. Rommelaere. Transfection with extracellularly UV-damaged DNA induces human and rat cells to express a mutator phenotype towards parvovirus H-1. Mol.Cell. Biol.(1984)324-328.

H. Braggaar, J.J. Cornelis, J.L.M. van der Lubbe and A.J. van der Eb. Mutagenesis in UV-irradiated simian virus 40 occurs predominantly at pyrimidine doublets. Mut.Res. 142(1985)75-81.

J.L.M. van der Lubbe, C.M. van Drunen, J.J.L. Hertoghs, J.J. Cornelis, J. Rommelaere and A.J. van der Eb. Enhanced induction of SV40 replication from transformed mammalian cells by fusion with UV-irradiated untransformed cells. Mut.Res. 151(1985)1-8.

F. de Foresta, J.J.L. Deleys-Hertoghs, J.J. Cornelis, B. Klein and J. Rommelaere. La transformation par le virus SV40 sensibilise les fibroblastes de peau humaine a l'action lytique de parvovirus H-1. Virologie, C.R.Soc.Biol. 179(1985)276-282.

G. Hilgers, J.J.L.Deleys-Hertoghs, J.J.Cornelis, B. Klein and J. Rommelaere. Reactivation du Parvovirus H-1 irradie aux Rayons gamma dans les cellules de patients atteins d'Ataxia telangiectasia et de la choree de Huntington. Virologie C.R.Soc.Biol. 179(1985)283-289.

C. Dinsart, J.J. Cornelis, M. Decaesstrecker, J.L.M. van der Lubbe, A.J. van der Eb and J. Rommelaere. Differential effect of ultraviolet light on the induction of simian virus 40 and a cellulair mutator phenotype in transformed mammalian cells. Mut.Res. 151(1985)9-14.

J.L.M. van der Lubbe, P.J. Abrahams, C.M. van Drunen and A.J. van der Eb. Enhanced indiction of SV40 replication from transformed rat cells by fusion with UV-irradiated normal and repair-deficient human fibroblasts. Mutation Research 165(1986)47-56.

G.J. Hilgers, P.J. Abrahams, R. Schouten, J.J.Cornelis, A.R. Lehmann, A.J. van der Eb and J. Rommelaere. Les cellules de patients atteints d'ataxia telangiectasia manifestent une capacité normale de réactivation radioinduite du virus HSV-1 endommagé. Virolgie C.R.Soc.Biol. 181(1987) 432-438.

P.J. Abrahams. A.A.M. van der Kleij, R. Schouten and A.J. van der Eb. Absence of induction of enhanced reactivation of Herpes simplex virus in cells from Xeroderma Pigmentosum patients without skin cancer. Cancer Res. 48(1988) 6054-6057.

J.L.M. van der Luhbe. Effects of ultraviolet irradiation on mutagenesis and induction of latent viruses in mammalian cells. Theses, Leiden 1987.

J.L.M. van der Lubbe, H.J.M. Rosdorff, J.L. Bos and A.J. van der Eb. Activation of N-ras induced by ultraviolet irradiation in vitro. Oncogene Research 3(1988)9-20.

J.L.M. van der Lubbe, H.J.M. Rosdorff and A.J. van der Eb. Homologous recombination is not enhanced in UV-irradiated normal and repair-deficiënt human fibroblasts. Mut.Res. 217(1989)153-161.

G. Hilgers, P.J. Abrahams, Y.B. Chen, R. Schouten, J.J. Cornelis, J.E. Lowe, A.J. van der Eb and J. Rommelaere. Impaired recovery and mutagenic SOS-like responses in Ataxia telangiectasia cells. Submitted to Mutagenesis 1989.

P.J. Abrahams, A.A.M. van der Kleij, R. Schouten and A.J. van der Eb. Absence of induction of enhanced reactivation of therpes simples virus in Xeroderma pigmentosum cells correlates with absence of skin cancer in XP patients. Molecular and Cellular Biology, submitted.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Carlsberg Laboratory
Department of Physiology
10, Gamle Carlsberg Vej
DK - 2500 Copenhagen Valby

Contract no.: BI6-E-168-DK

Head(s) of research team(s) [name(s) and address(es)]:

Prof. D. von Wettstein
Department of Physiology
Carlsberg Laboratory
10, Gamle Carlsberg Vej
DK - 2500 Copenhagen Valby

Telephone number: 01-221022 5225

Title of the research contract:

Chromosome pairing, crossing over and disjunction in human meiosis.

List of projects:

1. Chromosome pairing, crossing over and disjunction in human meiosis.

Title of the project no.: Chromosome pairing and disjunction in human meiosis

Head(s) of project: Prof. Diter von Wettstein

Scientific staff: Dr. S.W. Rasmussen
Cand. scient. B. Wischmann

I. Objectives of the project:

The assessment of the effects of radiation and radiomimetic agents on human meiosis requires a detailed knowledge at the ultrastructural and molecular level of the normal course of meiosis. The work carried out during the previous program will be continued and extended to include the characterization of meiosis specific structures at the biochemical level, an analysis of the long and short term effects of radiation on meiosis, a reconstruction analysis of the meiotic prophase in the male mouse and the human female as well as an investigation of the effects of chromosome pairing and crossing over on regular disjunction in polyploid species.

II. Objectives for the reporting period:

The proposal outlined in the contract has been followed with special emphasis on the ultrastructural characterization of the immediate effects of ionising radiation on meiotic prophase chromosomes.

III. Progress achieved:

Methodology Previous analyses of the short term effects of radiation on meiotic chromosomes from Bombyx mori spermatocytes have been impeded by the failure to obtain spread chromosome complements in sufficient quantities for a meaningful evaluation of the primary lesions caused by ionising radiation. A new preparation method was therefore developed which, instead of gravity settling of the lysed nuclei onto glass slides covered with a supporting membrane, included a centrifugation step during which the lysed nuclei were sedimented through a 0.5 M sucrose cushion directly onto membrane-covered copper or nickel mesh grids. With this method the spread chromosome complements adhered sufficiently well to withstand the subsequent fixation, washing and staining in ethanolic uranyl acetate and in lead citrate. The best preparations yielded 100–150 well spread complements per grid. For each absorbed dose (group B: 5 Gy; group C: 10 Gy; group D: 20 Gy; ^{60}Co, 1.17 Me eV, 1.35 Gy/min) ten animals were sacrificed on day 5, 7, 9, 11, 13 and 15 after irradiation at the beginning of the third larval instar, i.e. when the first cells at meiotic prophase appear. The spread nuclei were examined in a Zeiss EM 902 electron microscope. Even following an absorbed dose of 20 Gy the viability and development of the larvae were normal. The frequency of aberrations in the control group (1 translocation, 4 tetraploid and 2 partial tetraploid nuclei in a total of 1,570 nuclei) was constantly low from day 5 to day 15 and the observations were pooled in Table 1.

Results In most cases the complexity of the entangled zygotene complements prevents a detailed analysis in the electron microscope beyond an identification of the stage. From day 5 to day 15, 11% of the analysed control complements were at zygotene whereas only 0.5% of the nuclei in group B and none in groups C and D were at zygotene. At day 7 zygotene nuclei reappear in the spread preparations comprising 8% of all cells in group B, 11% in group C and 4% in group D. From day 7 onwards the frequency of zygotene nuclei varied between 15 and 24% in groups B and C and between 1 and 30% in group D. The latter, large variation probably reflects the small number of cells obtained for group D whereas the somewhat higher frequencies for groups B and C more likely represent a radiation induced change in the temporal progression of cells through the early meiotic prophase. Degenerating nuclei were frequent in preparations from group D but were seldom seen in spreads from groups B and D.

Five days after irradiation chromosome breakage and rearrangements were found in 27–33% of the spread complements in the three groups. In addition, tetraploid nuclei as well as nuclei in which the lateral components (LC) were longitudinally split with a morphologically normal central region between the two "half" LC's were present at frequencies between 10 and 17%. In contrast to the rare tetraploid nuclei in the control group (and in induced tetraploid larvae as described in the previous annual report) which contained a number of quadrivalents, the radiation induced 4n nuclei were devoid of quadrivalents. Most likely such polyploid nuclei originate by complete splitting of the LC's followed by formation of additional central regions between the divided LC's. It is uncertain whether this apparent chromosome doubling is accompanied by an additional round af DNA replication or whether the chromosomes contain a single chromatid each.

The most marked change from day 5 to 7 is the complete disappearance of LC breaks and the increase in the frequencies of tetraploid and partial tetraploid nuclei. From day 7 to day 15 the frequencies of aberrant nuclei gradu-

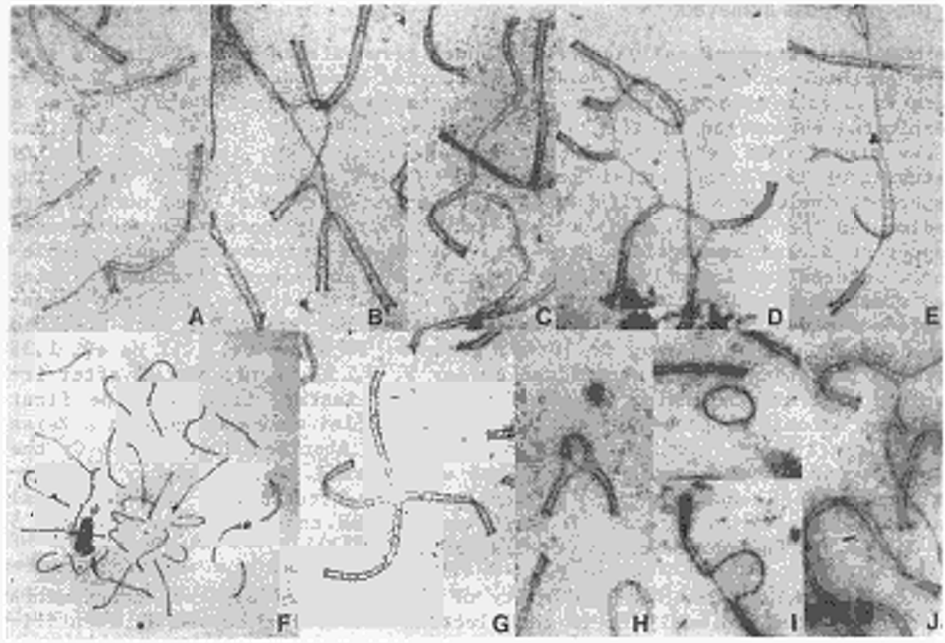

Figure 1. Radiation induced chromosomal rearrangements in Bombyx spermatocytes as visualised by the synaptonemal complexes.

A. Lateral component breakage. B. Double synaptonemal complex interlocking. D. Longitudinally split LC's with apparently normal central regions between the two "half" LC's. D. Translocation hexavalent with reversely paired segment. E. LC breakage in partially paired bivalent. F. Complete chromosome complement of normal pachytene nucleus. G. Translocation quadrivalent. H. Reversed loop pairing in an inversion heterozygote. I. Deletion and circular LC fragment. J. Short hairpin loop. A+B: control; C+E+F+G+J: 5 Gy; D+H+I: 10 Gy.

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ally decline in the B group, 99.2% of all complements being normal at day 15. The only detectable effect of radiation is the sevenfold increase in the number of nuclei with chromosomal rearrangements, mostly in the form of reciprocal translocations involving 2 chromosome pairs. A similar pattern is observed for group C except that the frequency of normal pachytene cells at day 15 only reaches 97.5%, 2.4% of the complements containing chromosomal rearrangements. These data show that even after irradiation with an absorbed dose of 10 Gy the gonads have the capacity of restoring a morphologically almost normal meiotic prophase. This is not the case in the D group. As can be seen from Table 1 the total number of cells obtained after spreading decreases from day 9 onwards. The frequency of nuclei with chromosomal rearrangements varies between 7 and 30%, the rearrangement more frequently being of the type illustrated in Figure 1D involving more than 2 chromosome pairs.

The various types of chromosomal rearrangements found after irradiation are shown in Figure 1. Altogether 1,138 nuclei with one or more chromosomal re-

arrangements were detected. Of these the vast majority were simple or complex interchange rearrangements formed by breakage and reunion of 2 or more chromosomes giving rise to translocation quadrivalents (Figure 1G) or chains or rings of several chromosomes (Figure 1D). In addition 24 deletions (Figure 1I), 4 inversions (Figure 1H) and 1 short interstitial hairpin (Figure 1J) were detected. Disruption of the LC's are occasionally found in normal nuclei at zygotene in most cases associated with resolving interlockings or intertwinings. These disrupted LC's appear stretched (Figure 1A) in contrast to the situation shown in the group B bivalent in Figure 1E in which the LC's appear dense and unstretched, the breakage most likely originating as a result of the irradiation.

Interlockings were frequent at zygotene and early pachytene in both control and irradiated nuclei from day 5 onwards, but were not quantified in the present analysis.

Table 1. The frequency (%) of pachytene cells with chromosomal aberrations after irradiation of the testes with absorbed doses of 0, 5, 10 and 20 Gy. (LC = lateral component of synaptonemal complex; SC = synaptonemal complex)

Day	Normal pachytene	Rearrangement	Tetraploid	Partial tetraploid	LC or SC breakage	Number of nuclei
0 Gy						
	99.5	0.1	0.3	0.1	0.0	1,570
5 Gy						
5	63.3	20.6	5.9	3.4	6.9	996
7	62.2	19.6	10.4	7.8	0.0	878
9	85.3	10.4	3.0	1.4	0.0	569
11	94.7	5.0	0.0	0.4	0.0	666
13	97.2	2.0	0.5	0.3	0.0	776
15	99.2	0.7	0.2	0.0	0.0	721
10 Gy						
5	62.7	21.9	3.5	6.5	5.3	711
7	64.0	14.7	18.7	2.6	0.0	526
9	79.2	16.0	3.6	1.2	0.0	497
11	91.4	7.3	1.0	0.3	0.0	464
13	97.2	2.4	0.4	0.0	0.0	565
15	97.5	2.1	0.3	0.1	0.0	905
20 Gy						
5	49.4	28.0	9.7	7.7	5.2	731
7	53.1	20.4	22.8	3.8	0.0	455
9	64.8	30.7	3.4	1.1	0.0	101
11	85.4	13.5	1.0	0.0	0.0	110
13	93.1	6.9	0.0	0.0	0.0	32
15	87.5	12.5	0.0	0.0	0.0	46

Discussion Previous work under this contract has described the meiotic prophase in considerable detail in the silkworm and in man and has shown that at the ultrastructural level there are only marginal differences in the basic processes as identified by the formation and behaviour of the synaptonemal complex. In both organisms a LC is organised in each chromosome, the LC attaches to the nuclear envelope and a chromosome bouquet forms as a prelude to synapsis and SC formation. In both organisms interlockings and intertwi-

nings occur during the chromosome movements required for precise synapsis, and later become resolved by breakage and precise rejoining of the involved chromosomes. In both organisms irregularities of pairing are extremely rare at pachytene, the homologues being held precisely in register by a continuous SC. It is therefore reasonable to assume that the effect of ionising radiation on meiotic prophase chromosomes and their LC's or SC's as revealed in the silkworm, reflects processes also occurring in the human testes and hence that the present data are relevant for the elucidation of the short term effect of radiation in general.

The correlation between the dose and the observed frequency of induced chromosome aberrations as measured by aberrant pairing behaviour of LC's during SC formation obviously depends upon the assumption that the SC formation faithfully mirrors the homologies of the members of the aberrant association. This assumption is only to some extent fulfilled. As described in our last annual report the frequency of quadrivalents initially formed during zygotene in autotetraploid Bombyx spermatocytes by the end of pachytene decreased to about half the original value. This transformation of multivalents into bivalents probably also occurs in the irradiated nuclei reducing the number of detectable aberrations at pachytene. Similarly, correction of pairing in inversion heterozygotes converting the reversed loop pairing into a stretch of nonhomologously paired straight SC may in part explain the very low frequency of detected inversions. The frequencies determined in the present analysis therefore represents a minimum estimate.

These results confirm the previous notion that radiation exposure of gonads leads to a temporary cessation of meiosis. After a period new spermatogonia mature and proceed into meiosis which then at least after moderate exposure appears essentially normal. It remains to be investigated to what extent the aberrations caused by irradiating early meiotic stages are due to chromosome breakage as such or to inhibition or failure of resolution of interlockings and intertwinings at zygotene.

The biochemical approach to identify enzymes involved in the resolution of interlockings has proceeded with the purification of DNA topoisomerase II from tissue culture cells of Drosophila and production of monospecific antibodies which cross-react with the corresponding Bombyx proteins. Immuno-gold labeling of meiotic cells with the antibody is in progress.

IV. Objectives for the next reporting period:

The analysis of the material described in the present report will be continued and the results published. The biochemical characterization of the synaptonemal complex, especially the role of DNA topoisomerase II in meiosis will be pursued by immuno-gold localization of topoisomerase II in the meiotic prophase nucleus.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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VI. Publications:

RADIATION PROTECTION PROGRAMME
Progress Report
1988

Contractor:

University of Aarhus
Ndr. Ringgade 1
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Contract no.: BI6-E-170-DK

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 6-125177

Title of the research contract:

The molecular basis for the interaction of radiation and carcinogens with the eukaryotic genome and the mechanism of repair. Studies on human and other eukaryotic cell cultures.

List of projects:

1. The molecular basis for the interaction of radiation and carcinogens with the eukaryotic genome and the mechanism of repair. Studies on human and other eukaryotic cell cultures.

Title of the project no.:

The molecular basis for the interaction of radiation and carcinigens with the eukaryotic genome and the mechanism of repair. Studies on human and other eukaryotic cell cultures.

Head(s) of project:

Ole Westergaard and Ole Frederik Nielsen

Scientific staff:

Anni Hangård Andersen, Bjarne Juul Bonven, Kent Christensen, Carsten Herskind, Eigil Kjeldsen, Palle Schelde Jensen, Klaus Hartnack Kristiansen, Steen Mollerup Kristensen, Tinna Ventrup Stevnsner, Boe Sandahl Sørensen, and Bo Thomsen.

I. Objectives of the project:

The aim of the project is to investigate the effect of ionizing radiation and carcinogens in specific regions of the eukaryotic genome with special reference to the humane genome, and to clarify the molecular mechanisms by which DNA damages are repaired.

II. Objectives for the reporting period:

Our investigations have been concentrated on the study of mechanisms leading to DNA damage when chromatin is exposed to ionizing radiation and the effect of scavengers on the processes. Furthermore, we have studied enzymes involved in repair and recombinational processes with special attention to the function of the sequence specific enzymes, type I and type II topoisomerases.

III. Progress achieved:

Radiation induced DNA damage on a specific eukaryotic gene isolated in its transcriptionally active chromatin form.

A biologically active eukaryotic chromatin system has been applied for radiobiological in vitro studies . The system consists of a single gene isolated in its transcriptionally active form. The gene is isolated in different chromatin complexities with transcriptionally active RNA polymerase molecules. The endogenous transcriptional activity has been used to study inactivation of the gene by ionizing radiation. From the inactivation curve, the number of critical lesions on DNA, which block transcription, has been estimated (1,2).

Inactivation by aqueous radicals

The sensitivity of the chromatin irradiated in a purified solution of nucleoli showed that direct ionization of DNA only contributed a neglegible fraction of the inactivation. This was confirmed by a radiation-chemical analysis which showed that the OH· radical was the major inactivation species and that the H· radical possibly was equally efficient. The contribution from the solvated electron e_{aq} was small if significant at all. In the purified solution the sensitivity was nearly the same in the presence of oxygen and in anoxia.

Inactivation by secondary radicals of OH scavengers

The radiation-chemical analysis involved the use of various radical scavengers. These scavengers only protected at low concentrations in 100% oxygen. At higher contrations and in anoxia they protected less than what should be expected from removal of OH· radicals. The low degree of protection was even more pronounced in anoxia. It was found that alcohols protect less than carboxylate ions.

The oxygen enhancement ratio

The larger extent of protection by the OH· scavengers in oxygen than in anoxia results in oxygen enhancement ratios lower than one, i.e. oxygen appears to be protective when the scavengers are present. The lowest value 0.28 was reached at concentrations of t-butanol higher than 0.3 M. In contrast, the sulphydryl compound 2-mercaptoethanol was more protective in anoxia than in oxygen. The protection in 100% oxygen at concentrations of 2-mercaptoethanol lower than 0.7 M might be explained by OH· scavenging of the SH compound.

Implications for inactivation of cells

It appears from the studies that secondary radicals of low molecular weight compounds may react with DNA and its constituents. The experiments show that inactivation of transcription by indirect effects could be explained by a hierachy of primary and secondary radicals. Radical scavengers reacting with the most important inactivating species may form secondary radicals which contribute to inactivation in the next place. In addition, species which give a small contribution to inactivation in the absence of scavengers may become relatively more important when the scavengers remove the major inactivating species.

Interaction of topoisomerases with chromatin; its implication for DNA repair and recombination.

In most biological systems DNA behaves as if it is topologically constrained, i.e. as if the two ends of the molecule are fixed. This property of DNA allows for higher order structures which may dictate biological functions. For example if the number of times two DNA strands are intertwined in a topological domain is diminished, i.e the domain is underwound, strand separation is favored. This will facilitate processes such as DNA replication, recombination, and DNA repair. Regulation of DNA topology is, thus, of critical importance to normal cellular functions. One of the principal means by which the cell accomplishes this is by the topoisomerases. These enzymes can be divided into two classes, type I and type II, both of which plays an important role in regulatory cellular processses. Thus, the bacterial topoisomerases are the primary target for a number of new very potent antibiotics, while the mammalian enzymes are the target for a number of important antitumor drugs.

During the last years we have studied mammalian type I and type II topoisomerases. We have shown that both classes of enzymes can operate on DNA in a highly sequence specific manner. Also, we have demonstrated that the the mechanism by which topoisomerases mediate antitumor activity is so that the action of antitumor drugs does not result from blocking a normal enzyme function but rather by subverting it in such a way as to render the enzyme to a lethal instrument. Thereby, the enzyme becomes a required cofactor for drug action. An important question raises from this concept: how do the drugs cause fragmentation of the newly synthesized DNA and do the fragments represent intermediates in the post-replication repair process (3-5)?

Genetical and biochemical studies have elucidated the roles of topoisomerases in replication, recombination, and DNA repair (1-6). The finding that the enzymes are the targets for several classes of drugs attests to their physiological importance. However, what is less clearly understood is why topoisomerases in some of their actions are functioning in a highly sequence specific manner. Since the binding preference for the recognition sequence is an intrinsic property of all eukaryotic topoisomerases, it seems plausible that the interaction of topoisomerases with their recognition sequences is important in highly conserved regulatory processes (5).

It is intersting that several human cell lines selected for resistance to DNA damaging agents do have elevated levels of topoisomerases and that cells from patients with high radiosensitivity show hypersensitivity to topoisomerase inhibitors. These observations support the idea that topoisomerases are involved in radiation damage. In order to investigate this correlation, we have recently isolated and characterized human cell lines being resistent to antitumor drugs (3-4).

Another reason for thinking that topoisomerases are important in eukaryotic DNA repair is that they may be the main target for the action of poly(ADP-ribose) transferase after DNA damage. This is based on the observation that massive poly(ADP-ribose) synthesis is the usual eukaryote response to DNA damage and that the modification results in inactivation of the enzyme. The modification of topoisomerase may lead to a change of the chromatin structure which ultimately might result in an increased accessability of the damaged DNA to repair enzymes (5).

IV Objectives for the next reporting period:

During the final period of the present contract we will continue our studies on the mechanisms which cause damage to DNA by exposure of transcriptionally active and inactive chromatin to ionizing radiation. Also, the involvement of eukaryotic topoisomerases in the processes of recombination and repair and the modulation of the function of these enzymes by chemotherapeutica will be studied extensively.

V Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

1. Herskind, C., and Westergaard, O. Variable protection by OH scavengers against radiation-induced inactivation of isolated transcriptionally active chromatin: the influence of secondary radicals. Radiation Research 114, 28-41 (1988).

2. Herskind, C. Sulfhydryl protection and the oxygen effect on radiation-induced inactivation of r-chromatin in vitro. Radiation Research 115, 141-151 (1988).

3. Kjeldsen, E., Bonven, B.J., Andoh, T., Ishii, K., Okada, K., Bolund, L., and Westergaard, O. Characterization of a camptothecin resistent human DNA topoisomerase I. J. Biol. Chem. 263, 3912-3916 (1988).

4. Kjeldsen, E., Mollerup, S., Thomsen, B., Bonven, B.J., Bolund, L., and Westergaard, O. Sequence-specific effect of camptothecin on human topoisomerase I DNA cleavage. J. Mol. Biol. 202, 333-342 (1988).

5. Kjeldsen, E., Bendixen, C., Thomsen, B., Christiansen, K., Bonven, B.J., Nielsen, O.F., and Westergaard, O. The influence of camptothecin on topoisomerase I interaction with genomic sequences. In Proceedings from the Second Conference on DNA Chemotherapy, New York, October 1988 (Ross, W. and Potmesil, M.,eds.). In press.

6. Andoh, T., Ishii, K., and Kjeldsen, E. Human DNA topoisomerase I mutant. In Protein, Nucleic Acid and Enzyme 33, 1784-1792 (1988).

THESES.

Kristensen, Steen Mollerup. Purification of topoisomerase I from Tetrahymena, human cells and wheat germ and studies on their DNA sequence specificity in the absence and presence of camptothecin. Thesis, Aarhus University, 1988.

Sørensen, Boe Sandahl. Eukaryotic topoisomerase II is associated with specific sites in the rDNA spacer of Tetrahymena thermmophila. Thesis, Aarhus University, 1988.

Jensen, Palle Schelde. Topoisomerase II: purification and characterization of the type II topoisomerase from calf thymus, Drosophila melanogaster, human tissue culture cells and Tetrahymena thermophila. Thesis, Aarhus University, 1988.

Hindkjær, Johnny Juhl. Production of monoclonal antibodies directed against human topoisomerase I. Thesis, Aarhus University, 1988.

Stevnsner, Tinna Ventrup. Interaction between DNA topoisomerase I and a specific binding sequence. Thesis, Aarhus University, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-E-206-GR

Greek Atomic Energy Commission
Nuclear Research Center
"Demokritos"
Aghia Paraskevi Attikis
GR - 153.10 Athens

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Zannos
Laboratory of Biological Dosimetry
Nuclear Research Cent. "Demokritos"
Aghia Paraskevi Attikis
GR - 153.10 Athens

Dr. G.E. Pantelias
Lab of Biological Dosim.
Nuclear Reseach Cent.
"Demokritos"
Aghia Paraskevi Attikis
GR - 153.10 Athens

Telephone number: 01-651.1360

Title of the research contract:

A new analysis of radiation-induced cytogenetic damage in human lymphocytes using the PCC technique, and its implications for biological dosimetry and the understanding of cell-cycle-dependent radiosensitivity fluctuations

List of projects:

1. A new analysis of radiation-induced cytogenetic damage in human lymphocytes using the PCC technique, and its implications for biological dosimetry and the understanding of cell-cycle-dependent radiosensitivity fluctuations.

Title of the project no.:

A new analysis of radiation-induced cytogenetic damage in human lymphocytes using the PCC technique, and its implications for biological dosimetry and the understanding of cell-cycle-dependent radiosensitivity fluctuations.

Head(s) of project:

Dr. G.E.Pantelias

Scientific staff:

G.Politis, M.D.
K.Sambani, Graduate Student

I. Objectives of the project:

1. The development of a sensitive biological dosimeter, based on the analysis of C-banded peripheral blood lymphocyte prematurely condensed chromosomes (PCCs), for the early assessment of radiation injury and the establishment of absorbed dose estimates in accidental overexposures.

2. To examine whether cell-cycle-dependent fluctuations in the fixation and/or repair of radiation-induced cytogenetic lesions are reflected by fluctuations in the expression and/or repair of potentially lethal damage (PLD).

3. To study the effects of hyperthermia on the induction and repair of radiation induced chromosome damage as visualized by the PCC technique.

II. Objectives for the reporting period:

A. To study the effect of araA and araC, two specific inhibitors of DNA polymerases alpha and beta, and of aphidicolin, specific inhibitor of DNA polymerase A, on repair of radiation-induced damage at the DNA, the chromosome and the cell level in plateau-phase CHO cells.

B. To examine the effect of BrdUrd incorporation on cell radiosensitivity as well as on the induction by radiation of DNA double strand breaks and chromosome damage in CHO cells.

C. To study the effect of pre-exposure to heat on the induction and repair of chromosome damage in plateau phase CHO cells using the PCC technique.

III. Progress achieved:

A. EFFECT OF DNA REPAIR INHIBITORS ON REPAIR OF RADIATION DAMAGE

Methodology

Experiments were designed to study the ability of compounds acting via inhibition of DNA polymerases such as araA, araC and aphidicolin, to inhibit repair of radiation-induced damage at the DNA, the chromosome and cell levels in plateau phase CHO cells. For the experiments at the chromosome level, the PCC technique was used. Repair of total DNA breaks was measured by the unwinding technique and repair of DNA double strand breaks by the neutral filter elution technique. The results obtained at these endpoints were compared with results of experiments evaluating inhibition of DNA replication, as well as with results of experiments measuring fixation of radiation-induced PLD by these compounds.

Results and Discussion

In agreement with the hypothesis that DNA polymerase activity is involved in cellular repair reactions, araA and aphidicolin strongly inhibited repair of radiation induced damage at the DNA and the chromosome level. Also, araC inhibited repair at these endpoints but only to a very limited extent. The relative inhibition of repair by these compounds was similar at the various endpoints studied. At the survival level araA effectively fixed radiation induced PLD resulting in survival levels corresponding to an exponential survival curve. On the other hand, araC and aphidicolin had only a small effect on cell survival. DNA replication was effectively inhibited by aphidicolin, moderately by araC, and even less by araA. These observations demonstrate that the efficacy of a polymerase inhibitor to inhibit DNA and chromosome repair does not always coincide with its ability to fix radiation induced PLD, or with its ability to inhibit DNA replication. This finding indicated that partly different biochemical pahtways may underlie PLD fixation, DNA repair inhibition and DNA replication inhibition.

B. EFFECT OF BrdUrd INCORPORATION ON RADIATION DAMAGE

Methodology

In order to characterize the relative contribution of increased DNA damage induction and increased PLD fixation in the halogenated-pyrimidine-induced radiosensitization, experiments were carried out at the DNA level, using non-unwinding DNA filter elution, at the chromosome level, using the PCC technique, and at the cell level. Mainly plateau phase cells were used as a biological system in order to enable parallel experiments at DNA, chromosome and cell level. Experiments at the cell level were also performed with exponentially growing cells and the results obtained were compared to those obtained with plateau phase cells.

Results and Discussion

Incorporation of BrdUrd into DNA, in the place of thymidine, sensitizes exponentially growing and plateau phase CHO cells to a subsequent exposure to low LET radiation. An increase in the amount of DNA and

chromosome damage induced per unit radiation dose was observed with increasing incorporation of BrdUrd into DNA that was quantitatively similar to the increase observed in the survival curve slope. Although sensitization was observed both in cells irradiated in the exponential as well as in cells irradiated in the plateau phase of growth, the degree of sensitization was significantly larger in exponentially growing cells for the same degree of thymidine replacement by BrdUrd in the DNA. It is hypothesized that this result indicates the possible importance of chromatin structure at the time of irradiation and/or the importance of chromatin conformation changes after irradiation in the expression of radiation induced potentially lethal damage in BrdUrd containing cells. BrdUrd incorporation affected both the slope and the shoulder width of the survival curve, and increased the induction of DNA and chromosome damage per unit absorbed dose. The increase observed in the survival curve slope was quantitatively similar to the increase observed in damage induction at the DNA and the chromosome level, suggesting a cause-effect relationship between these phenomena. Reduction in the shoulder width did not correlate with the increse in DNA and chromosome damage induction suggesting that different phenomena, probably related with enhanced fixation of radiation induced PLD in BrdUrd containing cells, underlie its modulation.

C. HYPERTHERMIA

Methodology

In order to study the effect of hyperthermia on the induction and repair of radiation induced chromosome damage, as a first stage experiments were designed to study the effects of heat (43 and 45.5 C) on chromatin morphology and nuclear organization, as visualized by PCC, in exponentially growing and plateau CHO cells. Experiments were also carried out with exponentially growing HeLa cells.

Results and Discussion

The results obtained indicate that exposure to heat drastically reduces the ability of interphase chromatin to condense and the ability of the nucleolar organizing region to disintegrate under the influence of factors provided by mitotic cells when fused to interphase cells. The fraction of cells with non-disintegrated nucleoli increased with increasing exposure time at 45.5 C and reached a plateau at almost 100% after about 20 min. Exponentially growing and plateau phase cells showed similar response. Recovery from the effects of heat on chromatin condensation and disintegration of the nucleolar organizing region depended upon the duration of the heat treatment. For exposures up to 15 min at 45.5 C, a gradual reduction in the fraction of cells with non-disintegrated nucleoli was observed when cells were allowed for repair at 37 C. However, only a very limited amount of repair was observed after a 30 min exposure to 45.5 C. The repair times observed at the chromosome level were similar to those reported for the removal of excess protein accumulating in chromatin or the nuclear matrix, suggesting a causal relationship between the two phenomna. It is proposed that nuclear protein accumulation on chromatin or in the nuclear matrix reduces the accessibility of chromatin to enzymes responsible for the phosphorylation reactions necessary for chromatin condensation and disintegration of the nucleolus.

IV. Objectives for the next reporting period:

A. BIOLOGICAL DOSIMETRY

Dose response curves for the most common radiation sources, dose rates, and qualities used, will be constructed by analysis of C-banded peripheral blood lymphocyte PCCs, and compared with those established using the conventional metaphase chromosome analysis.

B. MECHANISMS OF RADIATION ACTION

To study the effects of hyperthermia on the induction and repair of radiation-induced chromosome damage as visualized by the PCC technique.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Most of the experimental work involved in the achievement of the objectives for this reporting period was carried out in collaboration with Dr.G.Iliakis in the Laboratory of Experimental Radiation Oncology, Thomas Jefferson University Hospital, Department of Radiation Therapy and Nuclear Medicine, Philadelphia, PA 19107, USA.

VI. Publications:

G.Iliakis, G.E.Pantelias, R.Seaner and R.Okayasu, Comparative studies on repair inhibition by araA, araC and aphidicolin of radiation-induced DNA and chromosome damage in rodent cells: Comparison with fixation of PLD. International Journal of Radiation Oncology, Biology and Physics, in press (1988).

G.Iliakis and G.E.Pantelias, Effect of hyperthermia on chromatin condensation and nucleoli disintegration as visualized by induction of premature chromosome condensation in interphase mammalian cells. Cancer Research, in press (1988).

G.Iliakis, S.Kurtzman, G.E.Pantelias and R.Okayasu, Mechanism of radiosensitization by halogenated pyrimidines: Effect of BrdUrd on radiation-induced DNA and chromosome damage and its correlation with cell killing, Radiation Research, submitted (1988).

III F

BEWERTUNG VON STRAHLENRISIKEN UND OPTIMIERUNG DES STRAHLENSCHUTZES

EVALUATION OF RADIATION RISKS AND OPTIMIZATION OF PROTECTION

EVALUATION DES RISQUES D'IRRADIATION ET OPTIMISATION DE LA PROTECTION

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-227-E

Universidad Politecnica de Madrid
Departamento de Tecnologia Nuclear
C/José Gutierrez Abascal, 2
E-28006 Madrid

Head(s) of research team(s) [name(s) and address(es)]:

Prof. A. Alonso
Departamento de Tecnologia Nuclear
Universidad Politecnica de Madrid
c/José Gutierrez Abascal, 2
E-28006 Madrid

Telephone number: (1)262-62-00

Title of the research contract:

Off-site economic consequences of nuclear reactor accidents.

List of projects:

1. Off-site economic consequences of nuclear reactor accidents.

Title of the project no.: 1

Off-site economic consequences of nuclear reactor accidentes.

Head(s) of project:

Prof. Agustín Alonso

Scientific staff:

Assist. Prof. Eduardo Gallego
Jose E. Martín

I. Objectives of the project:

Development of a computer model for the assessment of the off-site economic risks derived from nuclear reactor accidents, based on a probabilistic approach.

The model will consider the direct costs of emergency countermeasures (evacuation, early relocation, food disposal) as well as those of long-term protective actions (food disposal, decontamination, temporary relocation, interdiction, permanent relocation). A model for the cost of radiation health damage will be also included.

The meteorological and socio-economical peculiarities of each site studied will be taken into account, by means of a flexible meteorological sampling scheme, and a data base compatible with the existing European grid.

II. Objectives for the reporting period: (1st January - 31th December, 1988)

* Completion of the implementation of models for the emergency and long-term protective actions.

* Development of the economic submodels of MECA (Model for assessing the Economic Consequences of Accidents).

* Design of the socio-economic data base, including the interface of the European grid with MECA.

* Collection, from an Spanish site, of the socio-economic data needed to perform a site-specific analysis with the new models.

III. Progress achieved:

1.- Methodology

Two sorts of inputs are needed by the model, called MECA, to assess the economic risks of accidents in the surroundings of a nuclear power plant:

a) Accident sequence specific inputs, such as probability; evacuation, relocation, food disposal, decontamination and interdiction areas; expected number of health effects.

b) Site-specific inputs, such as population distribution; farmland use; farm and live-stock production; industrial and commercial activity distribution; value of lands and properties.

The actual development of MECA is being made based on MACCS 1.4 (the MELCOR Accident Consequence Code System, recently developed by Sandia National Laboratories for the U.S. Nuclear Regulatory Commission), which will provide the accident specific inputs.

Site-specific data from Spain are being organized in a socio-economic data base compatible with the European grid system. Data are being collected at municipal level, when available. The geographical location and surface extension of each Spanish municipality are known from the National Geographic Institute. When data are only availsble at small region or provincial level, a sharing out proportional to areas is possible to obtain a finer resolution. Two computer programs allow the transformation of data sets from the Administrative grid to the European grid, -constituted by quasi-square elements of the same surface, and limited by meridians and parallels-, and to the calculational grid of circles and sectors around the reactor site.

The submodels included in MECA and the different economic items considered are the following:

* Population evacuation and/or temporary relocation costs, including transportation, housing and feeding, organization, salary and/or production losses.

* Food ban costs, at the market value of directly contaminated products.

* Decontamination costs, considering up to eigth possible decontamination levels and four different types of urban and rural surfaces. Production losses during decontamination are included.

* Interdiction costs, evaluated per unit area for farmland and per capita for urban areas.

* Population relocation costs, including production and value added losses during a transition period.

* Health effects costs, including reparations and medical care.

2. Results

The interface between MACCS and MECA is already completed and will transfer, for each accident sequence analysed, the data needed to define the protective actions model, the probability of the sequence, and the radiological health effects estimated. This interface provides to MECA detailed information about the countermeasures needed and expected health effects on each element of the grid.

The socio-economic data base contains data from Spain at three different levels:

* Municipal: Population (1986), surface, farm and live-stock census (1982).

* Provincial: Average annual farm and live-stock production (1979-1983), employment distribution by economic sectors.

* Local (around nuclear sites, not yet completed): average annual industrial and commercial production, value of lands and properties.

Computer programs for the transformation of data sets between the Administrative, calculational and European grid are ready. A first application has been carried out with population data.

MECA submodels, which are almost entirely programmed, combine, for each element of the grid, the information provided by MACCS with that from the socio-economic data base to assess the cost resulting from each accident sequence. Costs and accident sequence probabilities are combined to obtain CCDF's as economic risk estimates.

3. Discussion

The objectives for the reporting period are essentially reached. The extension of the socio-economic data base has been enlarged to all Spain with regard to population and farming, since data were available. On the contrary, detailed local data about industrial and commercial production around one nuclear site are not yet collected.

Programming of the model is almost completed, with the exception of submodels related with rural areas, which are being reelaborated according to the detailed data available from the farm and live-stock census (1982).

IV. Objectives for the next reporting period:

* Completion of MECA submodels for rural areas.
* Completion of site-specific data collection.
* Achievement of a first demonstration analysis of the model system, including uncertainty analysis of each submodel of MECA, using the Latin Hypercube Sampling (LHS) method.
* Elaboration of a final report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

1.- Alonso, A. and Gallego, E., A Model for the Calculation of the Off-Site Economic Consequences of Nuclear Reactor Accidents. In Proc. Joint CEC|OECD(NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, (Rome, Italy, 25-29th January, 1988). Report EUR 11408 EN.

2.- Gallego, E. and Martín, J.E., Aplicación del código MACCS al análisis de planes de emergencia. In Proc. XIV Annual Meeting of the Spanish Nuclear Society, (Marbella, Spain, 26-28th October, 1988).

3.- Gallego, E., Evaluación de las consecuencias económicas externas de los accidentes nucleares. Experiencia y perspectivas. In Proc. XIV Annual Meeting of the Spanish Nuclear Society (Marbella), Spain, 26-28th October, 1988).

4.- Martín, J.E. and Gallego, E., Distribution of the Spanish Population in the European Grid. Internal Report CTN-87/88. Madrid, November 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centro de Invest. Energéticas
Medioambientales y Technologicas
Division de Medicina
Avenida Complutense, 22
E-28014 Madrid

Contract no.: BI6-F-229-E

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F.R. Artalejo
Subdirec. Gen. Sanidad Ambiental
Centr. Invest. Energ. Medio. Tecno.
P° del Prado, 18 - 7° planta
E- 28014 Madrid

Telephone number: (91) 228.42.00

Title of the research contract:

Health effects of chronic exposure to low dose ionizing radiation on workers of the Spanish Nuclear Energy Institute.

List of projects:

1. Health effects of chronic exposure to low dose ionizing radiation on workers of the Spanish Nuclear Energy Institute.

Title of the project no.: B16-F-229-E

- Health Effects of chronic exposure to low dose ionizing radiation on workers of the Sapnish Energy Institute.

Head(s) of project:

- Dr. F. R. Artalejo.
  Subdirección General de Sanidad Ambiental.
  Paseo del Prado, 18-20, 7ª Planta.
  E-28014 Madrid.

Scientific staff:

- Dr. B. S. Fernández Murias, Dr. A. Rebollar, Dr. S. Castaño, Dra. C. Vázquez, Dr. Sainz Gancedo.

I. Objectives of the project:

a) to measure the mortality of the working population of the Spanish Nucelar Energy Institute (JEN).

b) to mesure the effect of chronic exposure to low-dose ionizing radiation.

c) to establish an automatized system for the epidemiologic surveillance of the effects of chronic exposure to radiation.

II. Objectives for the reporting period:

a) to finish the collection of exposure data (dosimetry data).

b) to collect outcome data. (National Institute of Statistics will -- provide data on mortality from JEN workers. We are carrying contacts with people responsible of the Institute on order to obtain this -- information without violating statistical confidentiality).

III. Progress achieved:

a) Collection of exposure data.

We have just finished the collection and automatization of all dosimetric data for all personnel of JEN who have been professionally exposed to ionizing radiation since 1956. We now have cumulative exposure data and exposure information desaggregated by calendar year and place of work for each worker.

b) Outcome data.

We have finished a consultation with the National Institute of Statistics and the National Institute of Social Security to collect information on vital status of each worker. At the moment we are carrying out a mail survey directed to the 3500 families of all workers of JEN to depurate and actualize some of these data. We hope to have this information at the end of march, and then begin the cross-checking of the data.

c) Analysis of results.

We hope to begin the analysis of data in May 1990.

IV. Objectives for the next reporting period:

a) Depurate outcome data.

b) Analyse the data set. We will make internal comparisons among workers of JEN obtaining rate ratios of mortality by exposure standardized by age and calendar time. We will also make external comparisons obtaining standardized mortality ratios adjusted by age and calendar time. The reference population will be the spanish population in 1979.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Dra. C. Gorostiza.
  DATA ANALYSIS CENTER.
  CIEMAT.

- Dra. M. Rodríguez Coma.
  DATA ANALYSIS CENTER.
  MINISTRY OF HEALTH.

VI. Publications:

- Health effects of low-dose exposure to ionizing radiation. Proceeding of the III Meeting of Environmental Health. Talavera de la Reina. 18 Nov, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-125-D

Gesellschaft für Reaktorsicherheit,
GRS mbH
Schwertnergasse 1
D-5000 Köln

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. A. Birkhofer
Gesellschaft für Reaktorsicherheit
Forschungsgelände
D-8046 Garching

Telephone number: (89) 32.00.40

Title of the research contract:

Methodology for probabilistic uncertainty analysis of computational assessments.

List of projects:

1. Methodology for probabilistic uncertainty analysis of computational assessments.

Title of the project no.:

Methodology for Probabilistic Uncertainty Analysis of Computational Assessments

Head(s) of project:

Dipl.- Math. E. Hofer

Scientific staff:

Mathematicians E. Hofer
B. Krzykacz
E. Nowak

I. Objectives of the project:

- Review of the spectrum of methods
- Application-oriented judgment of their relative merits and drawbacks
- Enhancement of their range of applicability
- Identification of unresolved issues

II. Objectives for the reporting period:

- Further development of DVA particularly with respect to the simultaneous treatment of uncertainty due to stochastic variability and uncertainty due to lack of knowledge;
- Extension of ICD to permit the inclusion of all given fractiles in the fitting of distributions;
- Inclusion of conditional pdfs in RED;
- Development of the first version of the package UST (uncertainty statements);
- Applications to accident consequence submodels and to the complete model.

III. Progress achieved:

1) Methodology

The set of program packages for uncertainty and sensitivity analysis is structured according to the main steps of a parameter uncertainty analysis:

	S T E P	Program Package
1	Compilation of potentially important uncertain parameters	
2	Maximum conceivable range of possibly applicable values	
3	Selection of subjective probability distributions to represent the knowledge base	ICD
4	Specification of dependences	ICD/RED
5	Propagation of the joint pdf through the model	RED DVA
6	Derivation of quantitative uncertainty statements for the model output	UST
7	Ranking of uncertain parameters (Sensitivity Analysis)	SAR
8	Presentation and interpretation of analysis results	RES

Several approaches to arrive at a quantitative uncertainty statement were implemented in the newly developed program package UST.

2) Results

UST derives quantitative uncertainty statements from the model outputs obtained for n parameter vectors generated with RED. Depending on the option selected by the user UST provides (respectively performs):

- distribution-free estimates of the desired u % fractiles of the subjective probability distribution of the model result;
- statistical tolerance limits (i.e.: v % confidence limits to the desired u % fractiles or intervals);
- tests for specific types of distribution;
- fractile estimates from fitted distributions;
- measures of association for different types of model output;
- estimates of the coefficients of a regression model and subsequent MC-Simulation with this model to derive fractile estimates from the resulting "empirical" distribution.

To facilitate the application of DVA its input structure was redesigned. Particularly the input of conditional distributions made this change necessary. Min- and Max-operation are now in the set of elementary DVA-operations. Furthermore, the histogram distribution

is included in the set of distributions that may be processed by DVA. In the course of a practical application it turned out useful to supplement the output options by information on the local effect of rebinning.

3) Discussion

UST is almost completed. RED, SAR and UST were successfully applied to practical assessment questions from the area of nuclear safety. Applications to accident consequence submodels and to the complete model are anticipated for the time period after completion of the internal testing phase.

The inclusion of conditional pdfs in RED was planned for this year, however, it proved impractical to perform such an extension of the package. At present, conditional pdfs (as one way of modelling dependence) may only be processed in DVA. Also the planned extension of ICD to permit inclusion of all given fractiles in the fitting of distributions could not yet be performed. Instead, efforts were concentrated on UST and improvements to DVA.

Details on the status of GRS-Program-Packages for uncertainty and sensitivity analysis were presented at the CEC MARIA-contractors' meeting in Karlsruhe, Oct. 1988.

IV. Objectives for the next reporting period:

- Completion of the main-frame computer version of each of the packages indicated under III.1, with the exception of DVA;
- Completion of the documentation of ICD, SAR, UST;
- Preliminary documentation of the package RES which is still to be developed.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- National Radiological Protection Board (UK), assessments department
- Kernforschungszentrum Karlsruhe (FRG), Project Nukleare Sicherheit

VI. Publications:

- NOWAK, E., HOFER, E., "A Programme Package to Support the Probabilistic Modelling of Parameter Uncertainties", in: Reliability of Radioactive Transfer Models, G. Desmet (ed.), Elsevier Applied Science Publishers Ltd., London and New York, (1988).
- KRZYKACZ, B., HOFER, E., "The Generation of Experimental Designs for Uncertainty and Sensitivity Analysis of Model Predictions with Emphasis on Dependences Between Uncertain Parameters", in Reliability of Radioactive Transfer Models, G. Desmet (ed.), Elsevier Applied Science Publishers Ltd., London and New York, (1988).
- KRZYKACZ, B., "MEDUSA 01" - Ein Programm zur Generierung von "Simple Random" - und "Latin Hypercube" - Stichproben für Unsicherheits- und Sensitivitätsanalysen von Ergebnissen umfangreicher Rechenmodelle, GRS-A 1496, Dez. 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-295-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. R.H. Clarke
NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: (235)831600

Title of the research contract:

Investigation of the relationship between lung cancer and radon in houses.

List of projects:

1. Investigation of the relationship between lung cancer and radon in houses.

Title of the project no.:

Investigation of the relationship between lung cancer and radon in houses.

Head(s) of project:

M C O'Riordan

Scientific staff:

J C H Miles, B M R Green, P R Lomas

I. Objectives of the project:

To determine the magnitude of the risk associated with radon exposure in the home by carrying out a case-control study of lung cancer incidence in relation to past and present household radon concentrations in SW England.

II. Objectives for the reporting period:

To devise a protocol for selecting lung cancer cases, hospital control and community control subjects, and to devise an appropriate questionnaire for the study. To commence interviewing the selected subjects, and to place passive radon detectors in their homes.

III. Progress achieved:

A protocol has been devised by the Imperial Cancer Research Fund for the selection of cases and controls. To be eligible for the study, cases and controls must be aged under 75 years, currently resident in Devon or Cornwall, and have lived in Devon or Cornwall for a substantial part of their lives - at least 20 years during the period between 5 and 35 years before the date of the interview. Cases must have been referred to hospital with a suspected diagnosis of lung cancer; any who subsequently turn out not to have the disease will ultimately be transferred to the control group or excluded, as appropriate. For each case, a hospital control is chosen matched by sex, age, and area of residence. As with the cases, the controls must have lived in Devon and Cornwall for a substantial period and, in addition, hospital controls must not have been admitted for a disease strongly associated with smoking, and must not be suffering from a condition that would preclude the taking of an adequate residential history. A questionnaire has been devised to allow smoking history, work history, diet, previous addresses and other relevant details to be recorded.

So far, 146 cases have been identified by ICRF. Some 73 subjects were ineligible, 61 because the residence requirement was not met and 12 who were too ill to give an interview. Ten subjects refused to take part, and 60 full interviews have been obtained. Interviews have yet to be carried out on three cases. No case has refused permission for radon detectors to be placed in his or her current home. Sixty-eight potential controls have been identified. Fifteen of these have been found to be ineligible, 14 because the residence requirement was not met, one who was too ill to be interviewed, and three who have refused to take part. Full interviews have been obtained for 46 controls and two have yet to be interviewed. Four controls have given a full interview but have refused to allow measurement of the radon level in their current homes.

For each eligible case or hospital control, the radon level in his or her home is being measured by NRPB using two detectors, one in the living area and one in the bedroom for a period of six months. In this reporting period, detectors have been distributed to the current addresses of 61 subjects. Radon measurements will also be made in addresses in Devon and Cornwall occupied by the subject between five and 35 years prior to his or her interview, i.e. approximately post-1950. On the average, excluding the current address, the subjects have 5.0 past addresses, just over three-quarters of which are in Devon or Cornwall. Of those past addresses in Devon and Cornwall, just under half are post-1950 of which in turn about 80% are complete enough to permit contact with the present occupier. This gives an average of 1.5 usable past addresses per subject. The present occupants of these addresses will be approached by NRPB and asked to place passive radon detectors in their homes.

IV. Objectives for the next reporting period:

To select appropriate community controls from the lists of Family Practitioner Committees, the Post Office address file, or electoral rolls. To expand the interviewing of cases and hospital controls so as to include a wider area in Devon and Cornwall. To continue interviewing cases and controls and placing passive radon detectors in their present and previous homes.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Imperial Cancer Research Fund
Cancer Epidemiology and Clinical Trials Unit
Oxford University
Gibson Building
The Radcliffe Infirmary
OXFORD OX2 6HE

Emeritus Professor Sir Richard Doll, Dr S C Darby, Dr P Silcocks.

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-112-B

Rijksuniversiteit Gent
Sint Pietersnieuwstraat 25
B-9000 Gent

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. A. Deruytter
Dosimetry Department
Laboratorium voor Kernfysica
Proeftuinstraat 86
B-9000 Gent

Telephone number: (091) 22.87.31

Title of the research contract:

Evaluation of the impact of the domestic environment on the population exposure to radon daughters.

List of projects:

1. Systematic analysis of the radon daughter equilibrium in houses.
2. Study of the behaviour and nature of radon daughter ions and clusters.
3. Investigation of the radon exhalation from building materials and soils.

Title of the project no.: 1

Systematic analysis of the radon daughter equilibrium in houses

Head(s) of project:

Dr.H.Vanmarcke

Scientific staff:

Dr.H.Vanmarcke, Lic.R.Van Dingenen, Dr.A.Poffijn, Dr.Ir.P.Berkvens*, Dr.R.Jacobs

I. Objectives of the project:

Systematic study of the physico-chemical processes determining the fate of the radon decay products in the indoor environment. Measurements will be performed in a representative number of dwellings. Special attention will be paid to the influence of the ambient aerosol on the internal radon-radon daughter equilibrium. In particular it will be investigated if the exposure of the public can be expressed in terms of radon concentrations rather than in terms of radon daughter concentrations.

II. Objectives for the reporting period:

- Continuation and extension of the study of uncertainties in radon daughter measurement induced by assuming a steady state situation in the indoor environment.
- An intercomparison of techniques and methodologies to determine the active and inactive size distributions of the radon decay products in the indoor environment has been carried out between the university of Göttingen, the NRPB and the university of Ghent.

* Now at ESRF Grenoble

III. Progress achieved:

Methodology

a) A program, based on the Monte-Carlo simulation technique, has been developped to evaluate uncertainties in radon daughter measurement arising from fluctuations in the parameters of the room model. The program has been used to determine the effect of entering a room and starting some activity, for instance, setting up a system to measure the radon daughter concentrations in the room.

b) An intercomparison with the university of Göttingen and the NRPB has been carried out to investigate the differences between our methodologies for determining the size distribution of the radon decay products.

Twenty-one measurements were performed during three days in a house with radon concentrations ranging from 700 Bq/m^3 to 1200 Bq/m^3. The active and inactive size distributions were measured simultaneously with three screen diffusion batteries, with a Berner impactor and with a differential mobility particle sizer. The aerosol concentrations were changed by buring a gas-stove or by burning some candles or by smouldering cigarettes.

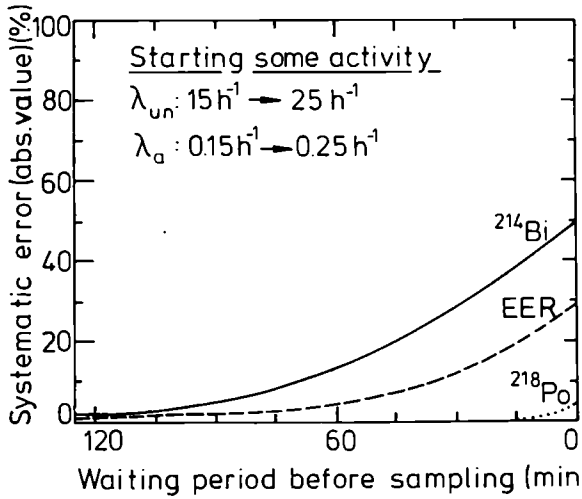
Each participant performed radon and radon daughter measurements. Simultaneously, nearly continuous measurements of the ventilation rate were performed by means of the release of N_2O tracer gas and observation of its decay with an infrared spectrometer.

Results and discussion

a) The details of the program were reported at the Lisbon meeting. Entering a room and starting some activity enhances the turbulence in the room and, as a consequence, enhances the deposition rates of the attached (λ_a) and unattached (λ_{un}) daughters. The effect on the internal radon-radon daughter equilibrium of a change of λ_{un} from 15 h^{-1} to 25 h^{-1} and of λ_a from 0.15 h^{-1} to 0.25 h^{-1} is illustrated in figure 1. Starting to sample the radon daughters half an hour after entering a room, for instance, will overestimate the ^{214}Bi concentration with 30%.

b) During the intercomparison, the ventilation rate in the room varied between 0.2 h^{-1} and 0.4 h^{-1}. The values noted during the night are at the high end, probably due to a greater difference between the outdoor temperature and the indoor temperature.

The unattached fraction was evaluated for each measurement. A value of about 10 % was found without aerosol sources in the room. The unattached fraction decreased below 5 % in the presence of aerosol sources. The different physical parameters of the radon decay products (attachment rate, deposition rate of the attached and unattached daughters, etc...) were also evaluated. Our results, together with the results of the other participants, will be published as a European report in the course of 1989.



1.1. The disturbance of the internal radon-radon daughter equilibrium due to a sudden change in the values of the deposition rates of the attached and unattached daughters. The systematic error is defined as the absolute value of the difference between the measured (simulated with the program) concentration and the steady state concentration, scaled by the steady state concentration. The systematic errors are given as a function of the period between disturbance and sampling.

IV. Objectives for the next reporting period:

- An investigation of the differences between the experimental techniques and the methodologies of the three groups participating in the intercomparison.
- The unattached fraction and the equilibrium factor will be studied in detail in view of the current debate whether recommendations and limits for reducing the exposure of the public are better expressed in terms of radon concentrations rather than in terms of radon daughter concentrations (EER).

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Isotopenlabor der Georg-August-Universität, Göttingen
  (Dr.J.Porstendörfer and Dr.A.Reineking, Burckhardtweg 2, Göttingen, F.R.G.)
- National Radiological Protection Board
  (Dr.J.Miles and Dr.J.Strong, Chilton, Didcot, Oxon OX11 ORQ, G.B.)
- Kernfysisch Versneller Instituut
  (Dr.R.de Meijer, Zernikelaan 25, Groningen, The Netherlands)

VI. Publications:

Vanmarcke H., Berkvens P. and Poffijn A. (1989) "Radon versus Radon daughters" Accepted for publication in Health Physics

Title of the project no.: 2

Study of the behaviour and nature of radon daughter ions and clusters

Head(s) of project:

Dr.H.Vanmarcke

Scientific staff:

Dr.H.Vanmarcke, Ir.C.Landsheere, Lic.R.Van Dingenen, Lic.K.Van Laere

I. Objectives of the project:

Since the unattached radon daughters do not have a single and fixed size, the simple equilibrium model that uses a single deposition rate and attachment rate, may not be sufficient. It will be tried to work out a more detailed model based on the knowledge of radon daughter ions and clusters, gained from theory and laboratory experiments. In particular it will be investigated how charge and size of the radon daughters depend on the environmental conditions like humidity, tracer gases, aerosol loading and turbulence.

II. Objectives for the reporting period:

To measure the deposition rates of the unattached radon daughters as a function of the degree of turbulence.
From the data the assumption of a single deposition rate for the three radon daughters is evaluated. An attempt is also made to derive a value for the exponent, n, in the expression of the eddy diffusivity in the theory of Crump and Seinfeld.

III. Progress achieved:

Methodology

The deposition rate of the unattached radon decay products is an important parameter of the room model, which describes the fate of a radon decay product in the indoor environment. The values reported in the literature for the deposition rate of the unattached decay products vary however over two orders of magnitude. In order to clarify some of this variability, we investigated the influence of the degree of turbulence on the deposition rate of the unattached daughters.
The experiments were carried out in our 1 m^3 radon chamber. The turbulence in the chamber was induced by closed circuit ventilation and/or by heat dissipation. The aerosol concentration in the chamber was less than 10 particles/cm^3, so that the fraction of radon decay products attached to the aerosol could be neglected. The deposition rates were derived from the radon concentration, measured by means of the Lucas method, and the three decay product concentrations, measured by means of α-spectroscopy. The turbulent diffusion constant, k_e, in the theoretical formula of Crump and Seinfeld for calculating the deposition rate, is examined by fitting the Crump and Seinfeld theory to our experimental results.

Results

According to Goldstein and Hopke, a diffusion coefficient of 0.07 $cm^2\ s^{-1}$ is used for the fitting of the theory of Crump and Seinfeld to the experimental ^{218}Po deposition rates. The expression for k_e is found to be proportional to λ_v^3 (ventilation) and $W^{3/2}$ (heat dissipation). This relationship was suggested by Friedlander in 1977. Another relationship, reported by Okuyama (1977), $k_e = \lambda_v^{3/2}$, does not agree with our findings.
In the literature different values are reported for the exponent, n, in the expression of the eddy diffusivity (n = 2 : Crump and Seinfeld; n = 2.6 : Van Dingenen; n = 2.7 : Okuyama; n = 3 : Friedlander). With our data it is impossible to discriminate between these values. In the optimization procedure we used the experimental determined value of Van Dingenen (see previous progress report).
We calculated with our expression the k_e values going with each experiment and applied them to compute the diffusion coefficients with the experimentally found values of the deposition rates. The results in the case of closed circuit ventilation are shown in figure 1 as a function of the lifetime of the ^{218}Po isotope and of the ^{214}Pb isotope in the radon chamber.

Discussion

We expect the diffusion coefficient to decrease with increasing lifetime. However, we only observe a difference between the two isotopes. The horizontal lines in the figure represent the weighted mean values of the experimental points. The difference in diffusion coefficient is to our opinion due to the chemical properties of the two elements. Further studies are needed to elucidate this important finding.

A significant difference in diffusion coefficient and as a consequence in deposition rate would explain some of the difficulties we found in fitting the room model to the data collected during our case studies in houses. Indeed, when we apply the deposition rate of the unattached ^{214}Pb to the unattached ^{218}Po, we systematically underestimate the measured radon concentrations.

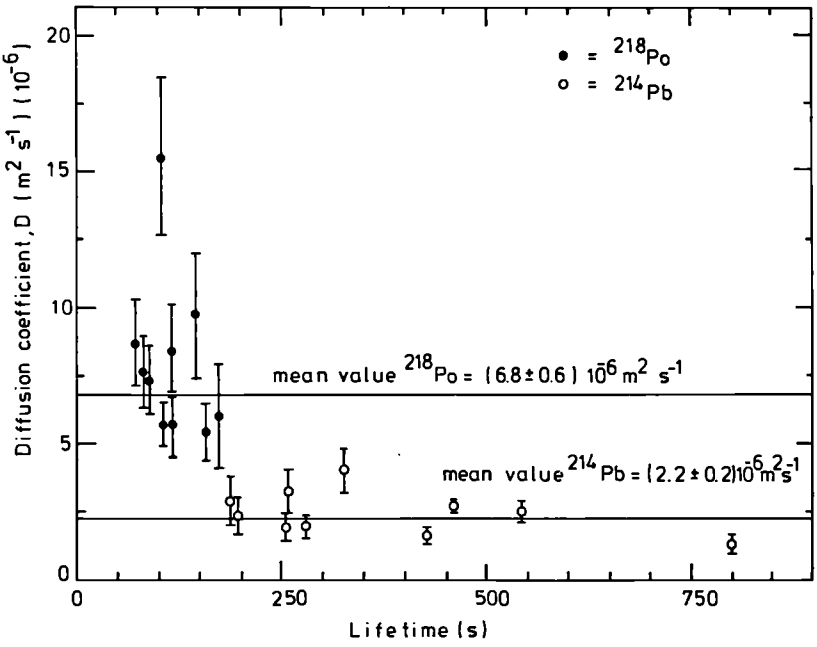


Fig.1. The diffusion coefficient of ^{218}Po and ^{214}Pb, corresponding with optimised k_e values, as a function of the lifetime of the isotopes in the chamber. A diffusion coefficient for ^{218}Po of 0.07 $cm^2 s^{-1}$ is assumed in optimizing the k_e values.

IV. Objectives for the next reporting period:

- To start a cooperation with Dr. Samuelsson (university of Lund) about his new technique of estimating retrospectively radon daughter exposure. This technique could become an important tool in epidemiological research.
- To evaluate the impact of the observed difference in deposition rate between the unattached ^{218}Po and the unattached ^{214}Pb on the results derived from the room model.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- CCR Ispra, Radiochemical Division
  (Dr.F.Raes, I-21020 Ispra, Varese, Italy)
- Lund University Hospital, Department of Radiation Physics
  (Dr.C.Samuelsson, S-22185, Lund, Sweden)

VI. Publications:

- Landsheere C. (1988) "Experimentele studie van de depositiesnelheden van de vervalprodukten van radon in funktie van de turbulentie" Thesis, RUG
- Van Dingenen R. and Raes F. (1988) "Determination of the sticking probability of H_2SO_4 on H_2SO_4-H_2O aerosols" Atmospheric Aerosols and Nucleation, Ed. P.E.Wagner and G.Vali, Springer-Verlag : Lecture Notes on Physics, 309, 23-26
- Van Dingenen R., Raes F. and Vanmarcke H. (1989) "Molecule and aerosol particle wall losses in smog chambers made of glass" Accepted for publication in J.Aerosol Sci., Vol.20, Nr.2

Title of the project no.: 3

Investigation of the radon exhalation from building materials and soils

Head(s) of project:

Dr.J.Uyttenhove

Scientific staff:

Dr.J.Uyttenhove, Dr.H.Vanmarcke, Dr.A.Poffijn, Dr.Ir.P.Berkvens*, Lic.J.Paridaens

I. Objectives of the project:

The main objective is to improve our knowledge of the radon source term and exhalation in Belgian houses. This is accomplished by a nation-wide survey, together with specific surveys to identify correlations with various parameters.
The contribution of radon and thoron exhalation from building materials will be investigated. A thorough theoretical treatment of the radon transport mechanism will accompany these investigations.

II. Objectives for the reporting period:

- In cooperation with the Belgian Building Research Institute (BBRI), a local survey was carried out in a region with potentially high radon concentrations.
- The thoron and radon exhalation of new types of phosphogypsum, sold in Belgium as building material, were measured and their impact on the population exposure of the public was estimated.

* Now at ESRF Grenoble

III. Progress achieved:

Methodology

a) A local survey has been carried out in a region where high radon concentrations are expected because of the geological properties of the ground. In 54 houses, selected ad random out of the telephone book, a radon detector was placed in the living room and if possible a second detector was placed in the cellar. The houses were monitored for one year.

b) New types of phosphogypsum are sold as building material in Belgium because industry has switched over from Moroccan phosphate ore to South African ore. The radioactive properties of two of these new types of phosphogypsum have been tested. From each type 30 plates of 0.295 x 0.210 m by 0.005 m and 30 plates of 0.295 x 0.210 m by 0.020 m were made. Four properties were assessed :
   - the specific ^{226}Ra and ^{232}Th activities,
   - the ^{222}Rn (radon) exhalation rate,
   - the ^{220}Rn (thoron) exhalation rate, uncovered,
   - the ^{220}Rn (thoron) exhalation rate, painted.

Results

a) The seasonal averaged radon concentrations in the living rooms vary between 30 and 4000 Bq/m^3 and between 35 and 14500 Bq/m^3 in the cellars. The concentration in the living room is in 10 % of the houses in excess of 150 Bq/m^3.

b) - The specific ^{226}Ra and ^{232}Th activities of the two types of phosphogypsum were measured by means of high resolution gamma spectroscopy :

   type 1 = ^{226}Ra : $75 \pm 5 Bq/kg$; ^{232}Th : $230 \pm 20 Bq/kg$

   type 2 = ^{226}Ra : $155 \pm 10 Bq/kg$; ^{232}Th : $160 \pm 15 Bq/kg$

   The thorium activity of the new types of phosphogypsum is much higher than the thorium activity of the Maroccan phosphogypsum. For the radium activity it is the other way about.

   - The ^{222}Rn exhalation rate was evaluated by closing the plates in airtight barrels and measuring the equilibrium concentration by means of the Lucas method :

   type 1 : 12 $Bq\ kg^{-1}s^{-1}$

   type 2 : 47 $Bq\ kg^{-1}s^{-1}$

   - The ^{220}Rn exhalation rate of the plates was determined by means of the ^{220}Rn decay product concentration in an airtight 1 m^3 chamber. The chamber was ventilated in a controlled way with outside air, in order to get sufficient aerosol particles inside the chamber. Each sample was measured 3 to 4 times. The reproducibility of the equilibrium equivalent thoron concentration is

about 25 %, which is entirely due to fluctuations in the aerosol concentration in the chamber. The thoron exhalation is, as expected from the short half-live of ^{220}Rn, independent of the thickness of the samples.

The effect of covering the entire surface of the plates with two layers of a common Latex paint was also investigated. The thoron exhalation after painting was 10 to 20 times lower.

Discussion

a) With our dose conversion factor of 20 Bq/m^3 = 1m Sv/y and assuming an occupancy factor of 80 %, we can conclude that 10 % of the people living in the area of our local survey are exposed to more than 6 m Sv/y.

We measured the radon concentrations in three houses close to the house with 4000 Bq/m^3 in the living room, or 160 m Sv/y. We found average concentrations of 3400 ± 200 Bq/m^3, 360 ± 20 Bq/m^3 and 165 ± 15 Bq/m^3 respectively, so that the highest value of our ad random survey is not an isolated case.

Mitigation measures, which consist mainly in installing a sub-floor ventilation system, are planned in the houses with a high radon concentration.

b) In order to evaluate the ^{220}Rn exhalation rates of the phosphogypsum plates, we converted the radon chamber results using the following "realistic" assumptions :
   - dimensions room : 4x5x3 m (60^3),
   - ventilation rate : 0.5 h^{-1},
   - walls and ceiling covered with the new types of phosphogypsum (74 m^2),
   - occupancy factor : 80 %.

The effective dose equivalent received by an occupant due to the thoron exhalation of uncovered gypsum is 1.8 ± 0.4 m Sv/y for type 1 and 0.9 ± 0.2 m Sv/y for type 2. Such a high dose, coming from one building material, can not be brought into agreement with the ALARA principle.

Painting the gypsum however lowers the ^{220}Rn dose by a factor of 10 to 20.

IV. Objectives for the next reporting period:

- Specific radon surveys will be carried out.
- The response of the Karlsruhe track-etch detector will be investigated as a function of the exposure (saturation of the detector).
- Mitigation measures are planned in a school and in a few houses. The effect of these measures will be evaluated.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Belgian Building Research Institute
  (Ir.P.Wouters, Ir.G.Demets, Av. Pierre Holoffe 21, 1342 Limelette, Belgium)
- Faculté Polytechnique de Mons, Département de Mines
  (Prof.Charlet, Rue de Houdain 9, 7000 Mons, Belgium)
- University of Groningen, Kernfysisch Versneller Instituut
  (Dr.R.de Meijer, Dr.L.Put, Zernikelaan 25, 9747 AA, Groningen, The Netherlands)

VI. Publications:

- Berkvens P., Kerkhove E. and Vanmarcke H. (1988) "Three-dimensional treatment of steady-state ^{222}Rn diffusion in building materials : introducing a practical modified one-dimensional approach" Health Phys., 55, 793-799
- Vanmarcke H. (1988) "Le radon : ce que c'est, d'où il vient et comment il peut nuire à notre santé" Bulletin de l'Association Universitaire pour l'Environnement, 22, 62-74
- Vanmarcke H., Poffijn A., Raes F., Eggermont G., Uyttenhove J., Berkvens P., Van Dingenen R., Bourgoignie R. and Jacobs R. (1988) "Radon in het leefmilieu" An. Belg. Stral., 13, 33-56

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Univ. degli Studi di Milano
Istit. di Fisica Generale Applicata
Via Festa del Perdono, 7
I-20122 Milano

Contract no.: BI6-F-174-I

Head(s) of research team(s) [name(s) and address(es)]:

Prof. U. Facchini
Istit. di Fisica Generale Applicata
Univ. degli Studi di Milano
Via Celoria, 16
I-20133 Milano

Telephone number: (02) 266.31.63

Title of the research contract:

Measurements of radon emission from soil of anomalous sites and investigation of radon concentration in the air of buildings in these areas.

List of projects:

1. Measurements of radon emission from soil of anomalous sites and investigation of radon concentration in the air of buildings in these areas.

Title of the project no.:

Measurements of radon emission from soil of anomalous sites and investigation of radon concentration in the air of buildings in these areas.

Head(s) of project:

prof. Ugo Facchini
Istituto di Fisica Generale Applicata - Universitā di Milano
via Celoria, 16 - 20133 Milano

Scientific staff:

M. Abruzzese, U. Galata, M. Lombardo, M. Magnoni
G.M. Marcazzan, G. Ravasini R.Rossi, G.Valli, A.Zambelli M. DeCarli G. Pacchioni.

I. Objectives of the project:

Realization of a number of detectors, capable of measurement of radon density in air.
Measurement of radon level in interiors, houses, cellars and wells, with particular research of anomalous sites, where radon level is even hundred times greater than usual.
Measurement of radon density performance versus time and possible correlation with metereological and geophisical factors.

II. Objectives for the reporting period:

Collection of data on radon level in anomalous sites and in particular: mapping of the area under study, follow up of data for long time period and study of correlation with metereological and geophysical factors.
Comparison with calibrated track etch detectors either in air and in soil; measurement of radon levels in water from wells.
Radium and Thorium concentration in rocks and minerals.

III. Progress achieved:

Methodology

A number of different approaches to radon level analysis has been followed:

a) Track-etch detectors
Track-etch detectors have been used for radon level density in air and in soil.
Recently the track-etch system has been developed in our Lab.: targets have been prepared and chemically treated; the lecture of alpha particle tracks is made by a spark device.

b) High resolution gamma spectrometry
the Laboratory disposes of a Ge-Li detector for gamma rays analysis; the calibration of the detector is made with known radioisotopes samples; rocks and soils have been investigated ; the content of radium and thorium is determined.
A second spectrometer, a thin germanium detector gives the low energy gamma spectrum and permits the identification of the long life Pb110.

c) Low resolution gamma spectrometry
A number of NaJ detectors 2"x2" and 3"x3" has been assembled; the detectors can be located either in dry wells or even directly immersed in water.
These instruments represent a very simple method for detecting radon daughters and its variation in time, where the randon density is sufficiently high.

d) Alpha particles detector for radon in water
The radon level in underground waters can be easily detected. The gasses dissolved in water are pumped and introduced in a small chamber where the walls are lined with fluorescent ZnS. A photomultiplier gives directly the alpha counts and the related radon level.

e) Alpha particles radon detector in air
The radon detector studied in our Laboratory is suitable for continuous recording of low densities of radon,thoron and decay products in air. The basic detector is a lucite disc of mm. 400 of diameter and 2 mm. thick; one faces of the disc is covered with a sensible ZnS layer; the layer is protected with a mylar foil, aluminized. The sensitive face of the disc is simply exposed to air and face to a photomultiplier; the pulses, resulting from the alpha particles scintillations are amplified and counted and the number is printed in a given time. The air sensitive volume can be

considered as a cylinder, with the disc diameter as a base and a height of few centimeters. The detector is simply put in air, either in interiors or in cellars or even in dry wells (Fig. 1).

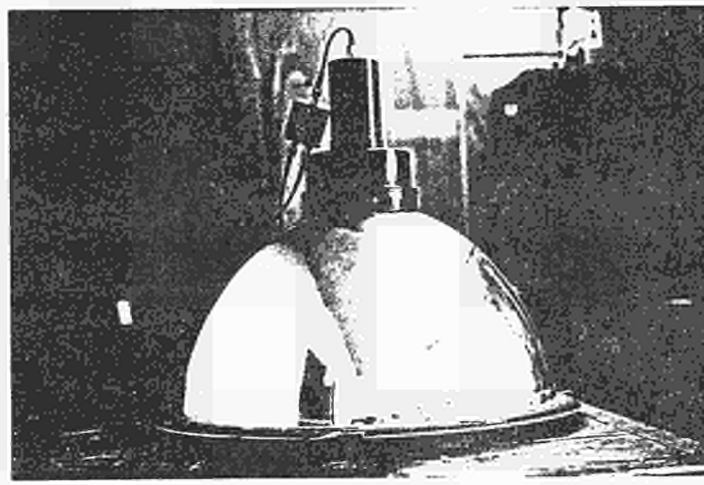


Fig. 1 - The portable alpha counter diameter 500 mm.

f) Calibration of the alpha detector
Our group partecipated to the 1987 CEC intercomparison of active and passive dosimeters, in Chilton (U.K.)
The results of the intercalibration show the following items:
when radon is measured in presence of a given aerosol concentration in air, as usually found in interiors, the pulses due to alpha particles originate in the given efficient volume around the ZnS layer.
When however the aerosol density is very low the radon daughters move toward the aluminized mylar surface of the detector and there plate out.
This fact is easily proved by taking out suddenly the detector from a rich radon atmosphere: 50-60% of counts persist and decay time corresponding to radon and thoron daughers decay times is observed.
Due to the attachment the sensible volume and the efficiency of detector are increased by a factor 2-3. The attachment

can be avoided with filters or by introducing suitable electric fields.
The detector gives reliable response in radon level measurements in interiors and can be used either for preliminary survey, as a portable instrument, or in a fixed station capable to give a follow up in time or radon levels.
Low radon levels of the order of 40 Becquerel/m^3 can be easily detected.

Results

Anomalous radon levels have been investigated either in buildings and in soils; three major factors can contribute to these levels:

o) High concentration of radium and thorium in rocks, sediments and building materials

oo) The presence of underground water with high concentration of dissolved randon

ooo) The presence in the underground of fractures where are active emissions of underground gasses.

The work of our group has considered all these factors, by examining the radon level in air and by taking into account the status of soil and rocks and, if necessary, of waters. The results we present in this report are grouped as follows:

a) rocks analysis: concentration of radium and thorium
b) underground water analysis: radon concentration
c) direct measurements in air in building and in dry wells

a) Radium and Thorium in rocks

The analysis is made with the Ge-Li detector, as said before, and the isotopic composition of the radioactive families is recognized by mean of the gamma spectra.
A number of rocks has been examined and in particular marbles and granites.
Table I shows a few results:
lowest radioisotope concentration is found in samples of marble; greater levels are found in granites: a few samples shows quite high concentration of radium and thorium.

TABLE I

Concentration of (becquerel/kg)	Ra226+and decay products	Th232+and decay products	K40
marbles	1-13	0-3	0-15
granites	12-350	12-350	300-1500

b) Radon in underground waters

A survey of radon levels in underground waters has been made; around 400 wells and springs have been examined in the Po Plain, in Lombardy, Veneto and Emily; average levels of radon of the order of 20-40Bq/l have been found; a survey done around the southern coast of Lago Maggiore evidenced 200-400 Bq/l of radon.
It is noticeable to observe as the presence of radon rich tap water can increase the radon level in the house and particularly in the cellars; in a number of high radon level cellars in Castellamonte near Ivrea, we have wells inside the cellar where the contents of randon in water results around 100 Bq/l.
In a few springs we have found radon levels as high as 1500 Bq/l.
In particular we have radon rich waters in proximity of the Novazza uranium mines and in a few sites in Valtellina and around the Monte Rosa.
The map of Fig. 2 shows the springs and wells examinated in Valtellina and Valchiavenna, Lombardy.

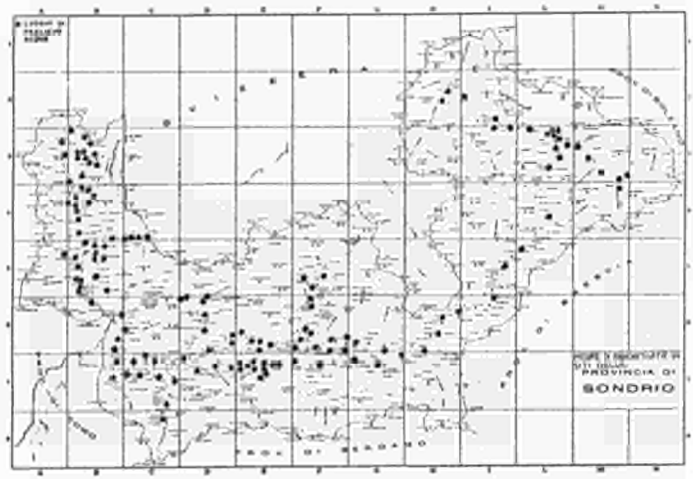


Fig. 2 - Map of wells and springs measured in the Sondrio province.

The radon level in buildings:
The measurements of radon level in interiors has been made as follows: first a survey with the portable alpha detector, then a measurement over integrated times with track etch detectors. In a few case a continuous measurement has been performed for days in order to esaminate the time behaviour of radon level. We report in Fig. 3 the results in the survey in Angera, a small town on the Lago Maggiore.
With the collaboration of the local USSL (Unità Socio Sanitaria Locale) a number of houses have been investigated; at the moment around 90. A group of houses, on the hills north of the town has been found with anomalous radon levels (A.R.L.).
In particular in the cellars and when holes or wells are there, high levels of radon, up to 4000 Bq/m^3 are observed.
The hills of Angera have a strongly fractured structure and the presence of uranium rich minerals.
Generally underground water is also in the neighnouring.
The randon rich houses are indicatively located on a geological fracture; the presence of the fracture have been evidenced by satellite observations.
In all houses monitored with alpha detector we have observed strong radon level variation, with major values in the night and lower at noon time; this behaviour is generally related to

atmospheric turbolence in daily hours. Fig. 4

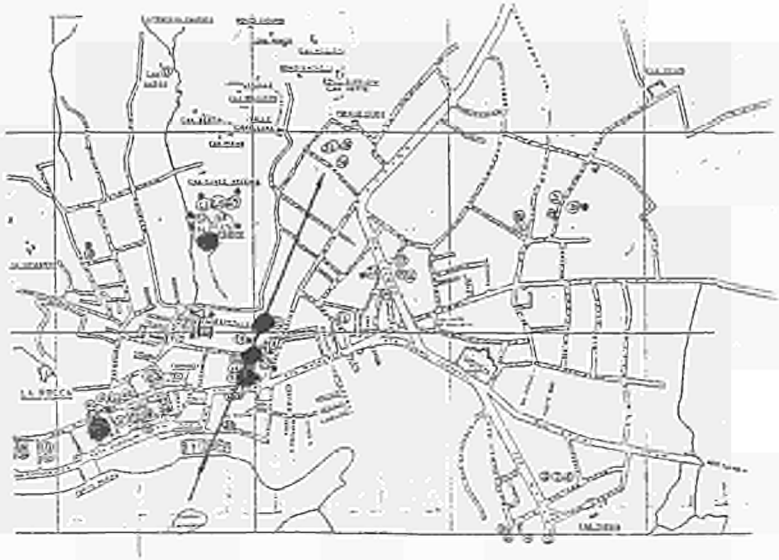


Fig. 3 - Map of survey in Angera interiors;open circles; survey points;black points: anomalous radon levels.

The Bertato' House

The Bertato's house was studied in years 80th by the ISPRA Center and then by our group.

Today the Bertato's house is recognized as one of the high level houses found in Angera.

Radon is emanating from a dry hole in the cellar of the house and there is indication of fractured soil and of underground water.

We measure the radon level in the cellar' hole during the years 1986-88, by printing hour by hour, the alpha pulses.

In fig. 5 the data are shown.

Beside the day-night oscillation we observe strong variations in the radon level by factor 20 or more; these variation can be related with metereological conditions; indicative results

show that in high pressure regime the radon level is lower and increases at low pressure days.
Average monthly level in the Bertato's house was found quite high (about 2.000-4.000 Bq/m^3)

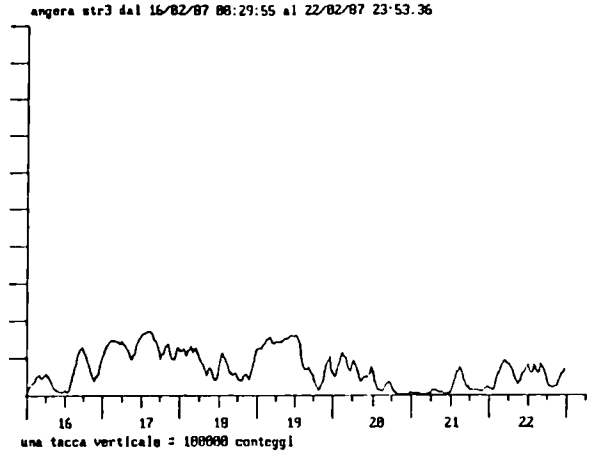


Fig. 4 - Day-night fluctuation in radon levels in the cellar of Bertato's house

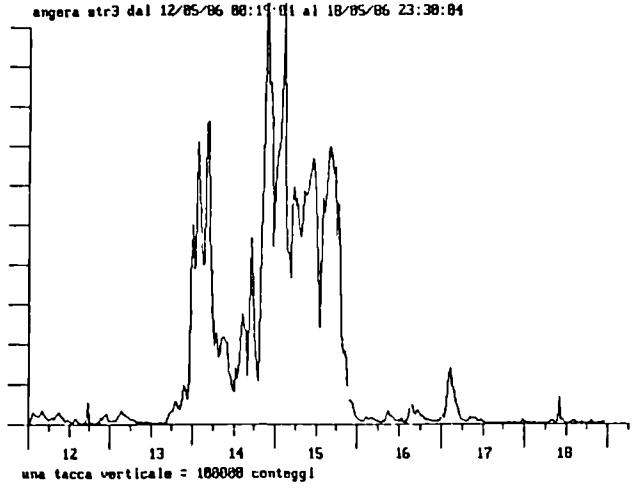


Fig. 5 - Strong variation in radon level in the Bertato's house may 1986.

Imagna' Station

A second monitoring station has been located in Bedulita, a site in Valle Imagna in the Prealpi bergamache where a strong fracture among different geological structures has been identified.
The fracture cut the mountain from 1000 metres height down the valley at 400 metres height.
At the lower altitude a number of houses has been found as anomalous radon level. In particular, near the Abruzzese's house, a strong radon density has been found in a dry well.
Average radon density in soil along the fracture has been measured by means of track etch detectors; we have the following data:

Altitude around 1000 metres	120-650 Bq/m^3
Altitude around 600 metres	670-1890 Bq/m^3
Lower altitude 400 metres	6.000-60.000 Bq/m^3

The measurements lasted 60 days in spring 1987.
The radon levels in the dry well have been monitored for six months either with alpha and with gamma detectors.
The radon behaviour is quite peculiar: a low level sequence lasting for a few days is followed by a sudden increase of radon level.

Discussion:

In sites and houses where A.R.L. are observed we have:
- presence of rich uranium rocks and minerals
- presence of underground fractures
- presence od underground water with high radon concentration
- interconnection of the house with underground, with wells either dry or with water
- diurnal variation of radon density in cellars and at the surface of the wells
- strong fluctuations in radon levels.

IV. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Presidio Multizonale Igiene Prevenzione;
  dott. Giuseppe Sgorbati - dott. Stefano De Crescenzo
  Via Juvara, 22 - Milano
- Unità Socio Sanitaria di Angera
- AGIP GEOTERMIA - Milano:
  dott. Roberto Carella

V. Publications:

U. Facchini, G. Ravasini, G. Sgorbati
Misure di radioattività nelle acque minerali e nelle acque sorgive.
Conferenza in Milano, Marzo 88

U. Facchini, P.M. Colombo, F.Villa
Le anomalie radon nelle acque profonde quale precursore sismico Terra, n. 04, 1987, 36-42.

In press: by the group of the Institute of Physiscs;
Radon emission from two wells in the Prealpi Lombarde; Radon concentration in spring and wells in Lombardy and Piedmont;
A map of radon level in interiors in the town of Angera;Radium and Thorium concentration in rocks and minerals.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre d'Etude sur l'Eval. de la
Prot. dans le Domaine Nucl., CEPN
B.P. n° 48
F-92263 Fontenay-aux-Roses

Contract no.: BI6-F-105-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Fagnani
CEPN
B.P. n° 48
F-92263 Fontenay-aux-Roses

Telephone number: (1) 4654.74.67

Title of the research contract:

Optimization of occupational exposure and implementation of the ALARA principle.

List of projects:

1. Occupational radiological protection optimization in the nuclear fuel cycle and in medical applications.
2. Methods for the practical implementation of the ALARA principle.

Title of the project no.: 1
OCCUPATIONAL RADIOLOGICAL PROTECTION OPTIMIZATION IN THE NUCLEAR FUEL CYCLE AND IN MEDICAL APPLICATIONS.

Head(s) of project:
LOCHARD J.

Scientific staff:
M. BENEDITTINI, A. FEYLESSOUFI, C. LEFAURE, J. LOCHARD

I. Objectives of the project:

The overall objective of the work is to develop case studies of optimization for occupational radiological protection in the nuclear fuel cycle and in the domain of medical activities. Two aspects are considered with a particular attention : the role of work management actions within dose reduction policies and the integration of risk distribution considerations in the optimization process.

II. Objectives for the reporting period:

The objectives for 1988 were the following :

- the updating of the occupational exposure data base in PWRs to include the most recent results as well as parameters allowing a better understanding of individual and collective dose evolutions in NPPs.

- the performance of ALARA case studies concerning the use of robotics and remote tooling to reduce occupational exposures in nuclear facilities.

III. Progress achieved:

The CEPN data base on occupational exposures in PWRs has been updated to include 1987 results. The data base includes now 121 reactors installed after june 30th 1974 which totalizes about 1170 reactors operating reactor years. The average annual collective dose per unit for the year 1987 is 2,57 manSievert that is a reduction of about 10 % with 1986 results.

Main efforts during this year have been devoted to the evaluation of robotics and remote control actions to reduce exposures in nuclear installations in an ALARA perspective. Apart from the development of a methodological framework to assess both direct extra protection costs and indirect operational cost savings, specific case studies have been performed. The first one is related to the evaluation of alternative automation options to manipulate mixed oxide fuel rods in the quality control shop of a fuel fabrication facility. The second one deals with the evaluation of the effectiveness of remote operators and control systems for the shot peening of steam generator tubes. The third one is an evaluation of remote control and robotic actions for the plugging of steam generator tubes.

IV. Objectives for the next reporting period:

The next period will be devoted to :

- the updating of the occupational exposure data base in nuclear power plants.

- the performance of ALARA case-studies concerning the shielding of equipments at the conception of nuclear facilities as well as the use of robotics and remote tooling to reduce exposures related to maintenance works in NPPs.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

BENEDITTINI M., LOCHARD J. Expositions professionnelles dans les réacteurs à eau pressurisée : comparaison internationale de quelques indicateurs globaux entre 1975 et 1987. CEPN - R - 132, Janvier 1989.

LOCHARD J. "Cost-effectiveness of robotics and remote tooling for occupational risk reduction at nuclear facilities" in : Man Machine Interface in the Nuclear Industry, Proceeding of an International [IAEA/CCE/OCDE-AEN] Conference, Tokio, Japan, 14-20 february 1988, IAEA, Vienna 1989.

LEFAURE C., LOCHARD J., BLAIN A. - "Evaluating Remote Control and Robotics Actions in NPPs in an ALARA Perspective : Lessons from the Evolution of Steam Generator Tube Plugging Technique in France". in : Radiation Protection Optimization : Advances in Practical Implementation, Third European Scientific Seminar, Madrid, Spain 12-14 September 1988, CCE, 1989.

Title of the project no.: 2

METHODS FOR THE PRACTICAL IMPLEMENTATION OF THE ALARA PRINCIPLE

Head(s) of project:

LOMBARD J.

Scientific staff:

LOCHARD J., LOMBARD J.

I. Objectives of the project:

(i) to develop a simple, general framework for future ALARA studies ;
(ii) to demonstrate the use of this framework by applying in examples studies.

These objectives will be achieved by reviewing the difficulties which have arisen in the practical implementation of ALARA, and suggesting and applying methods by which these difficulties may be resolved. The review is to be carried out by reference to studies reported in the literature and to work in progress at CEPN and NRPB. The examples chosen will concern a range of practices involving radiation exposure, including but not confined to, the nuclear industry. In suggesting methods to resolve these difficulties and in deriving the general framework, particular attention will be paid to indicating the capabilities of the various decision-aiding techniques (eg. multi-attribute analysis, cost benefit analysis...) and to identifying the areas where judgements are required by those responsible for taking ALARA decisions.

II. Objectives for the reporting period:

The objectives for 1988 were to consolidate the work of the previous years, to prepare a draft of the final report and furthermore to perform a preliminary study on the monetary valuation of the occupational man-Sievert for reference values in the french installations.

III. Progress achieved:

The early part of the project concentrated on two aspects, development of the basic structured approach of the ALARA Procedure and case studies of the application of this approach. In the last two years the work has covered the develompent of other structured ALARA elements, viz. ALARA Audits and Predictive ALARA Plans. This year papers have been published showing the interplay of these various elements, how they can be used in practice and some of the problem areas, particularly that of collecting relevant dosimetric data. The outline structure of the project report produced in 1987 has been modified to reflect this latter work and drafting of the report has been completed this year.

During 1988 the CEC Third European Scientific Seminar on Radiation Protection Optimisation took place in Madrid and provided a focus for the presentation of our work. Project staff from NRPB and CEPN were heavily involved in organising the Seminar and were responsible for 30 % of the papers.

During the forthcoming final year of the project the report will be rewied and finalized.

The content of the report is the following :

1. Foreward

SECTION I - INTRODUCTION

2. Introduction
   - 2.1. Concepts underlying ALARA
   - 2.2. The role of ALARA in radiological protection
   - 2.3. The ALARA procedure
   - 2.4. Misconceptions about ALARA

3. The ALARA procedure - a general overview
   - 3.1. Definition of the problem
   - 3.2. Identification of factors and options
   - 3.3. Pre-selection of options for analysis
   - 3.4. Quantification of options
   - 3.5. Comparison of options
   - 3.6. Sensitivity analysis
   - 3.7. Presentation of the results
   - 3.8. The final decision

SECTION II - THE ALARA PROCEDURE

4. Structuring the problem
   - 4.1. Identification of problem
   - 4.2. Identification of relevant factors
   - 4.3. Identification of protection options
   - 4.4. Selection of feasible protection options

5. Evaluation of protection costs
   - 5.1. Costing procedure
   - 5.2. The assessment of costs over time
   - 5.3. Interaction between protection and other costs

6. Radiological detriment to health
   - 6.1. Factors to be quantified
   - 6.2. Time distribution of doses

6.3. Probabilistic risk
6.4. Others factors that are relevant to the radiological health detriment

7. Other relevant factors
   7.1. Non-radiological risks
   7.2. Non-health detriment
   7.3. Social factors

8. Data collection
   8.1. Dose modelling
   8.2. Task-specific dosimetry

9. Evaluation of α and β
   9.1. The value of α
   9.2. The value of β

10. Decision-aiding techniques
    10.1. Cost-effectiveness analysis
    10.2. Cost-benefit analysis
    10.3. Multi-attribute utility analysis (decision analysis)
    10.4. Multi-criteria outranking analysis
    10.5. The choice of the appropriate decision-aiding technique

11. Sensitivity analysis
    11.1. Factors and options
    11.2. Models and data base
    11.3. Value judgements

SECTION III - IMPLEMENTING ALARA IN RADIATION PROTECTION PROGRAMMES

12. Implementing ALARA in design and operations
    12.1. Introduction
    12.2. Commitment to ALARA
    12.3. Management systems
    12.4. ALARA data base
    12.5. ALARA criteria

13. Implementing ALARA in strategic decisions.

ANNEXES : CASE STUDIES

IV. Objectives for the next reporting period:

The aim for 1989, is to carry out the necessary redrafting work to produce the final report. This will take into account the CEC's Third European Scientific Seminar on Radiation Protection Optimisation - Advances in Practical Implementation, 12-14 September, Madrid.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

NRPB
Chilton, Didcot, Oxon
OX 11 ORQ
England

VI. Publications:

PJ. SAUNDERS, JR. CROFT, AD. WRIXON, J. LOMBARD, J. LOCHARD, "Methods for the Practical Implementation of the ALARA principle : the CEPN/NRPB Joint Project", Third European Scientific Seminar on Radiation Protection Optimization, Madrid 12-14 Sept. 1988.

P. HUBERT, P. PAGES, "ALARA and Radioactive Materials Transportation", Third European Scientific Seminar on Radiation Protection Optimization, Madrid 12-14 Sept. 1988.

J. LOMBARD, P. HUBERT, P. PAGES, "ALARA and Waste Disposal", Third European Scientific Seminar on Radiation Protection Optimization, Madrid 12-14 Sept. 1988.

J. LOMBARD, R. COULON, A. DESPRES, "An ALARA Approach to the Radiological Control of Foodstuffs following an Accidental Release", Risk Analysis, Vol 8, n° 2, 1988.

JR. CROFT, J. LOCHARD, "Statut of Achievements Reached in Applying Optimisation of Protection in Design and Normal Operation of Reactors", The Application of Optimisation of Protection In Regulation and Practice, Proceedings of an NEA Meeting 16-18 March 1988, Paris.

J. LOCHARD, JR. CROFT, "Key issues in the Implementation of ALARA in Operations", Journal of Radiological protection, Vol. 8(2), 1988.

Report
J. LOMBARD, "Proposition de valeurs de référence de l'homme-rem pour l'exposition professionnelle", Rapport CEPN n° 136, Avril 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Centre d'Etude sur l'Eval. de la
Prot. dans le Domaine Nucl., CEPN
B.P. n° 48
F-92263 Fontenay-aux-Roses

Contract no.: BI6-F-207-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Fagnani
CEPN
B.P. n° 48
F-92263 Fontenay-aux-Roses

Telephone number: (1) 4654.74.67

Title of the research contract:

Comparison of methodologies for risk management applied to nuclear and non-nuclear industrial activities.

List of projects:

1. Comparison of methodologies for risk management applied to nuclear and non-nuclear industrial activities.

Title of the project no.:

COMPARISON OF METHODOLOGIES FOR RISK MANAGEMENT APPLIED TO NUCLEAR AND NON NUCLEAR ACTIVITIES.

Head(s) of project:

Dr FAGNANI F.

Scientific staff:

FAGNANI F., LE GALES C., HUBERT P., LOMBARD J., OUDIZ A., PAGES P., SCHNEIDER T.

I. Objectives of the project:

(i) to put into perspective the methodologies aiming at the management of the radiological risk and other industrial risks.
(ii) to demonstrate the methodological convergences of the two fields, based on case studies in the non radiological risks.

It is intended to apply the methodological structure currently developped in the radiological field under the name of ALARA to various non-radiological risk sectors : chemical carcinogens and hazardous material transportation.

II. Objectives for the reporting period:

The objectives for January 1-December 31 1988 period were as follows :

- Methodological development on the scientific grounds of TLVs, and on the question of dealing with uncertainty in dose response relationship (normal operation risk management).
- Methological development on the assessment of detriments other than immediate health effects and applicability of the previously developped risk criteria and assessment tools (hazardous material transportation risk assessment).
- Case study analysis of the industrial exposure of benzene and PCB in electricity transformers accidents.
- Case study analysis on the city of Lyons for the development of decision aiding tools.

III. Progress achieved:

3.1. Methodological aspects

Quantitative risk assessment of chemical carcinogens.

A synthesis of the work previously done on the dose response relationship for carcinogens allowed a comparison between the actual threshold limits values (TLVs) for acrylonitrile, ionizing radiations, asbestos, benzene and vinyl chloride showed that the life time risk of cancer due to one year of exposure ranges from 0.1 10^{-4} (lower bound for MVC) to 15.10^{-4} (upper bound for asbestos). The risk associated with the ionizing radiation limit is within the range of the others. Both the width of the range and the position of the radiation limit were far from being obvious. Both advocates for a quite easy application of a common ALARA framework.

An uncertainty analysis has been made on TCDD dose response relationship in support of the PCB case study.
A book has been issued, together with the French Medical Research Institute (INSERM). It deals both with the risk assessment and management of carcinogen substances. Together with other contributions, the previously performed case studies have been incorporated.

Accidental events and hazardous material transportation

Pragmatic models for pollutant transfer in river and groundwater have been set up and applied to the Grenoble area. More generally the methodological framework for implementing those models was defined. Data aquisition methods and definition of the proper risk indices were also defined. The latter question led to an important work on the selection of pollution damages that are relevant for decision making.

Common methodological framework

At this step of the project, it might be interesting to provide a first outlook on the results that begin to arise from the comparative exercise. As a matter of fact, all the skills developped in the radiation protection field have proven to be usefull. This is particularly true for the assessment tools, where, for example in the Grenoble case study, the aquatic transfer models and data banks set up for radiological survey purposes were readily usable. This is also true of the general ALARA step by step scheme and of the decision aiding tools. However in the latter case, as stated in the last progress report, it is generally the most sophisticated ones which are required.

The difficulties in applying ALARA in non radiological areas are rather due to the lack of the basic elements which are usually taken for granted when starting an ALARA analysis in the radiological field. The first one is the monitoring of individual exposures, which, however imperfect, has always been performed in the nuclear industry. In many industries it is not at all the case and the ALARA approach cannot therefore be grounded on actual practices or way of thinking. The same apply to some of the basic concepts such as collective doses. While the man-Sievert especially when restricted to a given occupational activity is a meaningful notion for most people in radiation protection, it is not the case with "man-ppm". Therefore it appears that when describing and defining the ALARA methodology, it was not paid enough attention to some of the pre-requisites of any ALARA study.

3.2. Case studies

Occupational risk in the chemical industry

The case study on benzene has been completed (Rapport CEPN n° 140). It involved an enquiry in order to reconstruct the benzene system in France. This system is quite complex

since 750 000 t of benzene are used every year as an industrial product. Its production involves the coal and petroleum industry and its use involves the chemical industry where it is an intermediary product for many purposes. Not all the workers in those industries are exposed to benzene, but, since benzene is a component of motor spirit, occupational exposures are not limited to activites dealing with benzene as such. Out of those surveys it was established that about 70 000 workers are exposed to benzene, out of them 1 600 are submitted to more than 1 ppm. As in previous case studies a critical analysis of the dose response relationship allowed to assess that the life time risk for a 35 years work time exposure to 1 ppm would range between 0.12 10^{-3} and 17 10^{-3} lethal leukemia. As the total collective exposure lies between 10 000 and 50 000 man-ppm, the total occupational risk would therefore range between 0,03 and 24 deaths per year, a figure to be compared with the 2 to 14 deaths attributed to benzene among city dwellers. Reducing the environmental risk is possible but at high cost (roughly equivalent to 1M $ per man-Sv). The decisional analysis is quite difficult since measures such as catalytic exhaust pipe are multipurpose measures.

On the PCB transformer, most of the work has been rather methodological and has been quoted above.

Transportation of hazardous materials

The methods set up about the Lyons study have been applied to the case of the road transit through the French city of Grenoble. They proved to be easily applicable. The results showed a quite lower risk than in Lyons. About 0,08 deaths is the yearly mathematical expectation of the risk, and it is associated with 1 chance out of 300 of having a catastrophic accident. As stated supra, the case study led to development of river and underground water transport models. They showed that a pollution accident can have heavy (although not fatal for man) consequences. 1 t of phenol would prevent use of water for alimentary purposes up to 200 km down stream. Pollution accidents are still scare but much more frequent (1 chance out of 12 every year) than catastrophic accidents (rapport CEPN n°142). In both cities, interviews have been conducted in order to know better how decision makers balance catastrophic versus less dramatic fatal accidents, and fatal accidents versus pollution accidents.

IV. Objectives for the next reporting period:

The case studies will be completed, namely the study on the management of the fate of PCB transformers and hazardous material transportation in cities. In both cases, the stress is now on the risk management aspect.

The synthesis of the work will be performed. The boundary between what can be said to be a common methodological approach in nuclear and non nuclear field, and what is not, will be defined, and common data, models and methods will be pointed out. Lessons will be drawn for the application of ALARA to non nuclear materials. Also the non nuclear field analysis will provide some inputs to the general improvement of ALARA methodology, especially when consequences other than health effects or when accidental events are to be dealt with. Connected research directions will be pointed out.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

A. OUDIZ, C. LE GALES, "Prévention des cancers professionnels. Problèmes et perspectives", Co-édition INSERM/DOIN, 1988, 33 pp.

Y. BONVALOT, "Evaluation des différentes méthodes de prédiction du risque due à l'exposition à un cancérogène chimique. Application au problème de la 2, 378 TCDD.", Mémoire soutenu le 16 octobre 1987, Y. Bonvalot, Paris, 1988.

P. HUBERT, J. LOMBARD, A.OUDIZ, P. PAGES, "Comparison of threshold limit values for different carcinogens", in Seminar on Applications, Perspectives and Limitations of Comparative Ris-Assessment and Risk-Management, Nice, Sept. 26-30, 1988.

Y. BONVALOT, A. OUDIZ, HUBERT P., ABENHAIM L., "Determination of carcinogenic threshold limit values using the tumorogenic dose rate 50% (TD 50%), in Seminar on Applications, Perspectives and Limitations of Comparative Ris-Assessment and Risk-Management, Nice, Sept. 26-30, 1988.

L. ABENHAIM, Y. BONVALOT, F. FAGNANI, A. OUDIZ, "The Value of Probabilistic Modelling for the Estimation of Carcinogenic Risk at Low Doses. Application to 2,3,7,8 TCDD", in Seminar on Applications, Perspectives and Limitations of Comparative Ris-Assessment and Risk-Management, Nice, Sept. 26-30, 1988.

P. HUBERT, P. PAGES, "Risk Management for Hazardous Materials Transportation : a Local Study in Lyon", in 1st European SRA Conference, Laxenburg 10-11 November 1988.

T. SCHNEIDER, JP. MOATTI, "Contribution of economic analysis for prevention of risk from toxic carcinogens : the french experience", Symposium on Management of Risk from Genotoxic Substances in the Environment, Stockholm, October 3-5, 1988.

Reports

A. OUDIZ, F. FAHRI, J. LOMBARD, "L'évaluation du risque dans la filière benzène", Rapport CEPN n° 140, Décembre 1988.

J. BRENOT, A. DESPRES, JP. DEGRANGE, P. HUBERT, P. PAGES, "Trafic des matières dangereuses sur l'itinéraire pilote de l'agglomération de Grenoble. Evaluation du risque. Rapport final", Rapport CEPN n° 142, Décembre 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Laboratorio Nacional de Engenharia
e Tecnologia Industrial, LNETI
DPSR - Azinhaga dos Lameiros à
Estrada do Paço do Luminar
P-1699 Lisboa

Contract no.: BI6-F-208-P

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.P. Galvão
DPSR
LNETI
Estrada Nacional 10
P-2685 Sacavém

Telephone number: (1) 255.49.81

Title of the research contract:

Evaluation of the population exposure to radon in the vicinity of uranium mining facilities.

List of projects:

1. Evaluation of the population exposure to radon in the vicinity of uranium mining facilities.

TITLE OF THE PROJECT Nº:

Assessment of population doses from radon inhalation in areas with technically enhanced concentrations of natural radioactivity and development of mathematical models for characterizing source terms.

HEAD(S) OF PROJECT: M.M.G.R.Teixeira
L.Canelas, as leader of the contribution from Departamento de Ciências e Engenharias do Ambiente, Universidade Nova de Lisboa
(DCEA/UNL).

SCIENTIFIC STAFF:

A.O.Bettencourt	L.Canelas (DCEA/UNL)
M.M.R.Teixeira	A.Brogueira
M.C.Faísca	M.M.Brito
	N.Nascimento

I. OBJECTIVES OF THE PROJECT:

To assess the exposure of the population critical groups to radon and to determine the contribution of the radium processing wastes and uranium tailings to this exposure. To study the influence of some meteorological parameters.

To implement a mathematical model for predicting the atmospheric dispersion of radon.

II. OBJECTIVES FOR THE REPORTING PERIOD:

To pursue the survey of indoor ^{222}Rn concentrations in the houses already under study and to enlarge it to other dwellings from this granitic region. To start measurements of outdoor Rn concentrations and to evaluate its contribution to indoor concentrations in a contaminated zone.

Installation of the anemograph and starting of its operation. Sampling and analysis of local meteorological data. First runs with the model.

PROGRESS ACHIEVED

1. Methodology

The survey programme for the determination of radon concentrations in Portuguese dwellings continued at the surroundings of an abandoned radium salts factory (Barracão) and it was enlarged to some towns and villages from the granitic region of Beira Alta. Indoor radon measurements were also performed in a non granitic region, to establish reference levels.

Solid nuclear track detectors were used as passive dosemeters and were exposed for 1-2 months.

A preliminary study of radon emanation from the main building materials used in the types of surveyed houses, was also performed. For this study, the LR-115 dosemeters were placed on the surfaces of different materials, sealed inside a polythene bag, and removed after different exposure times. The same building materials were also analysed by Ge(Li) gamma spectrometry.

Concerning atmospheric dispersion no local meteorological data could yet be sampled, due to difficulties in the emplacement of the anemograph. The mathematical model is being adapted to LNETI computers.

2. Results

Concerning indoor radon concentrations, a total of 1150 measurements were performed during this period. The geometric mean for each village or town, as well as the range of the mean values observed in the surveyed houses, are presented below:

REGION	Nº of houses per locality	Indoor radon concentration (Bq m^{-3})	
		geometric mean ($x10^2$)	range ($x10^2$)
Barracão	7	9.4	1.7 - 29
Granitic	23	1.1	0.6 - 2.5
	11	1.5	0.7 - 7.2
	8	1.9	1.1 - 4.1
	30	2.6	0.4 - 12.6
	14	1.4	0.7 - 4.9
	8	2.5	0.8 - 5.1
Non granitic	58	0.4	0.1 - 1.4

The highest radon concentrations were found in the houses near the old radium salts factory; the resulting effective dose equivalent, for this critical group, is of the order of 5 x 10 $mSv.a^{-1}$. The overall mean observed for the granitic region is 1.7 x 10^2 $Bq.m^{-3}$ with a amximum local mean of 2.6 x 10^2 $Bq.m^{-3}$ at the town of Guarda.

Mean effective dose equivalents around 9 $mSv.a^{-1}$ for the granitic region and 2 $mSv.a^{-1}$ for the non granitic one have been calculated.

Concerning radon emanation from different building materials, the values observed for some of the most significant ones, after an exposure of 100 days, were:

brick	- 1320 alpha tracks cm^{-2}
cement	- 880 alpha tracks cm^{-2}
sand	- 520 alpha tracks cm^{-2}
granitic stone	- 6500 alpha tracks cm^{-2}

The installation of the simulation model into LNETI computers is almost achieved.

3. Discussion

The results obtained up to now in the indoor radon concentration survey, clearly show three different levels of activity: the highest one due to the influence of the wastes from the radium salts factory; the two others due to different geological constitution of the soils (granitic and non granitic).

The relative influence of soils and building materials to indoor radon concentrations appear to depend on the considered region. The results obtained were presented at the "Conferência Nacional de Física", Aveiro 26-29 September 1988.

In what concerns modelling there has been a delay in the installation of the anemograph and therefore no local meteorological data could yet be sampled.

IV - OBJECTIVES FOR THE NEXT REPORTING PERIOD

Continuation of the on-going indoor radon survey and its enlargement to the whole granitic region. Comparison of the results with measurements to be performed i other regions from the country.

Outdoor radon concentration measurements, in order to calculate its contribution to radon inhalation by the critical groups, as well as sampling of meteorological data and mathematical simulation of outdoor radon concentrations.

V - OTHER RESEARCH GROUP(S) COLLABORATING ACTIVELY ON THIS PROJECT [NAME(S) AND ADDRESS(ES)]:

Departamento de Ciências e Engenharia do Ambiente
Universidade Nova de Lisboa
Quinta da Torre
P-2825 Monte da Caparica

VI - PUBLICATIONS:

M.Conceição Faísca, M.Manuela Ribau Teixeira
Influência dos Materiais de construção nas concentrações de radão medidas no interior de habitações.
Conferência Nacional de Física, Aveiro, September 1988

M.Manuela Ribau Teixeira, M.Conceição Faísca
Radão no Interior de habitações
Conferência Nacional de Física, Aveiro, September 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Risø National Laboratory
DK-4000 Roskilde

Contract no.: BI6-F-175-DK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H.L. Gjørup
Health Physics Dept.
Risø National Laboratory
DK-4000 Roskilde

Telephone number: (2) 37.12.12

Title of the research contract:

Shielding factor calculation for plume radiation

List of projects:

1. Shielding factor calculation for plume radiation.

Title of the project no.:

Shielding Factor Calculation for Plume Radiation

Head(s) of project:

Per Hedemann Jensen

Scientific staff:

Søren Thykier-Nielsen

I. Objectives of the project:

To develop a computer model for calculation of the protection by indoor residence against gamma radiation from a passing radioactive plume. Important parameters such as building structure dimensions, window apertures, plume/building geometry and photon energy will be identified by parameter studies. Representative shielding factors for typical European houses will be calculated.

II. Objectives for the reporting period:

Shielding factors for single-family houses and simple blockhouses have been calculated for:

- -different plume/building geometries
- -different window aperture sizes

An improved method for describing the influence of window apertures has been developed and the results have been compared to corresponding calculations with the Monte Carlo code MCNP.

III. Progress achieved:

1. METHODOLOGY

The mathematical model for calculating the indoor radiation dose from a gaussian plume has been further developed and improved regarding the influence of window apertures. Building types are described as a composite of cubic boxes, and attenuation and scattering in the walls and floors is accounted for in the numerical integration of the point kernel over the plume volume.

2. RESULTS

Variation of plume/building geometry

For a traditional Danish single-family house the plume/building geometry was varied. Two different atmospheric stability categories (Pasquill D and F), two different heights (0 m and 100 m), four different downwind distances and two different crosswind distances were used. Calculated shielding factors are shown below for a photon energy of 0.68 MeV and for a detector position in the center of the house.

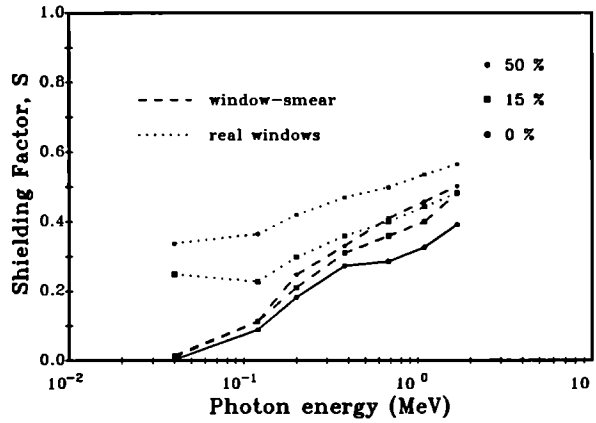
Downwind distance (meter)	Pasquill D	Pasquill F
(crosswind distance=0 m)	(plume height=0 m)	
200	0.04	-
500	-	0.03
1000	0.06	0.06
10000	0.09	0.05
(crosswind distance=0 m)	(plume height=100 m)	
200	0.22	-
500	-	0.20
1000	0.15	0.19
10000	0.09	0.11
(crosswind distance=500 m)	(plume height=0 m)	
10000	0.09	0.04

Variation of window fraction

To account for the poor shielding of windows, the outer wall thickness is in the model decreased to an effective thickness that wil depend on the fraction of outer wall area covered by windows. This "window-smearing" method gives too low shielding factors at low photon energies. Therefore, the model has been further developed so the real window apertures can be described.

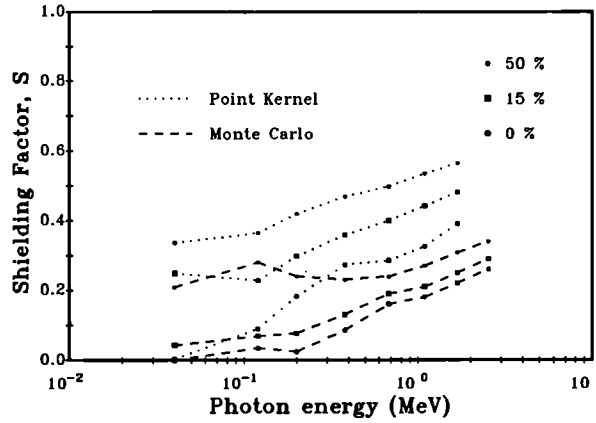
For a semi-infinite plume shielding factors were calculated for a simple blockhouse with 14% and 50% of the outer wall

area covered by windows. The results are shown in the Figure below as a function of photon energy. The mass thickness of the outer wall is 21 gram per square centimeter.



Monte Carlo calculations

As the point kernel method has some limitations, Monte Carlo calculations have been made for the simple blockhouse, and the results are shown in the Figure below. The corresponding point kernel results are shown for comparison.



3. DISCUSSION

The "window-smearing" method gives misleading results at low photon energies and will therefore be rejected. A temporary conclusion is that the point kernel method gives results that - compared to the corresponding Monte Carlo results - underestimate the indoor protection from plume radiation. An adjustment of the build-up factor to correct this discrepancy will, if possible, be developed.

IV. Objectives for the next reporting period:

Calculations of shielding factors for different housing conditions with variation of the following parameters:

-distance to and dimensions of neighbouring buildings
-window fraction and plume geometry

Calculation of shielding factors for typical European housing conditions.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

"Shielding Factor Calculations for Plume Radiation", Status Report, MARIA-Contractors Meeting, Karlsruhe, October 20-21, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Imperial College of Science
Exhibition Road
GB- London SW7 2AZ

Contract no.: BI6-F-228-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. A.J.H. Goddard
Mechanical Engineering Dept.
Imp. Coll. of Sc. and Techn.
Exhibition Road
GB- London SW7 2AZ

Telephone number: (1) 589.51.11

Title of the research contract:

Experimental studies on aerosol transport processes in dwellings using inactive tracer techniques.

List of projects:

1. Experimental studies on aerosol transport processes in dwellings using inactive tracer techniques.

Title of the project no.: BI6-F-228-UK

Experimental studies on aerosol transport processes in dwellings using inactive tracer techniques

Head(s) of project: Prof. A.J.H. Goddard
Mechanical Engineering Department
Imperial College of Science Technology and Medicine
London SW7 2AZ UK

Scientific staff: Dr. R.J. Cannell, Ms J. MacCurtain, Mr. N. Qadir

I. Objectives of the project:

To carry out experimental work aimed at a better understanding of the infiltration and transfer of fine particulate (aerosol) material within dwellings. To generate data relating to infiltration, deposition upon surfaces, mechanical ingress and transport, and resuspension. The research will take advantage of an ultra-violet scanning technique for measuring aerosol deposition patterns on surfaces. The initially stated objectives for the project include further calibration studies, studies on an experimental house and trial external infiltration studies using a dust generator.

II. Objectives for the reporting period:

Completion of commissioning of the environmental test chamber; studies of deposition and resuspension within the test chamber; initial measurements within a test house, using the ultra-violet scanning technique.

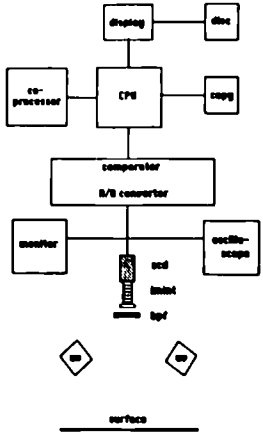
III. Progress achieved:

Under the parallel contract BI6-F-108-UK, a computer model has been developed which permits the estimation of the protection afforded by dwellings against the ingress of radioactivity in particulate form and the subsequent deposition and other transport processes of this particulate material within dwellings. Data for such a model is lacking; data is inadequate for infiltration, deposition, resuspension and the mechanical transport of the particulate materials into dwellings. The aim of this contract is to help provide the necessary data.

Collaboration with the Risø Laboratory in Denmark has been maintained. This collaboration resulted earlier in measurements of deposition velocity, for ambient Be-7 and Cs-137 bearing aerosols, within a typical Danish house.

At Imperial College the aim is to obtain data relevant to dwellings for the behaviour of mono disperse particles. The development, at the Imperial College Reactor Centre, of means of labelling mono disperse silica particles with activeable tracers, was extended to the labelling of these aerosols with UV-fluorescent tracers. This has permitted the use of non invasive scanning techniques to estimate deposition on surface with the aid of the DIADEM system.

The development DIADEM system is illustrated in diagrammatic form in the accompanying figure.



KEY: CCD charge-coupled device camera
uv ultra-violet lamp
disc mass storage
CPU data acquisition processor
copy graphics printer
imint micro-channel plate image intensifier
bpf narrow bandpass interference filter

DIADEM development system for non-invasive scanning of aerosol deposition on surfaces

The inclusion of customised micro-channel plate image intensifier between the lens and the CCD camera now provides an increased gain of up to 10^4, with a possibility of detection below 1 μg cm^{-2} of tracer surface loading. The calibration of the system, which must be carried out with known surface loadings, has been investigated with particular reference to problems with calibration near the limits of detection. The system has now been used inside a dwelling for further studies for the mechanical ingress of deposited aerosol material through the movement of occupants. In addition the system has been employed, in collaboration with UKAEA Harwell, in a study of the depletion of deposited aerosol on outdoor roads, by resuspension due to vehicular traffic.

The 2m cube aerosol test chamber has now been commissioned; the flow inside the chamber has been characterised and deposition experiments with mono dispersed aerosol, on the deposition of aerosol on a vertical surface, have been carried out with the aid of the DIADEM scanning system. The chamber has now been modified so that the DIADEM system may be mounted to scan floor or wall surfaces so that, in the same experiment, measurements may be made of relative deposition, on various types of surface.

Initial design work has been undertaken for the planning of experiments to elucidate the infiltration attenuation of aerosols.

IV. Objectives for the next reporting period:

Use of the DIADEM system, in conjunction with the aerosol test chamber in studying the deposition of mono dispersed aerosols on various types of surface, with attention being paid to deposition on both vertical and horizontal surfaces. Collaborative studies with the Risø establishment into the deposition of aerosols within dwellings.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Risø National Laboratory, Denmark (J. Roed)

Environmental Sciences Division, AERE Harwell (Dr. K. Nicholson)

VI. Publications:

Cannell, R.J. and Roed, J. Relationship between indoor and outdoor aerosol concentration following the Chernobyl accident.
Protection Dosimetry, 21. 1987. 1, 3, pp 107-110.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Imperial College of Science
and Technology
Exhibition Road
GB- London SW7 2AZ

Contract no.: BI6-F-108-UK

Head(s) of research team(s) [name(s) and address(es)]:

Prof. A.J.H. Goddard
Mechanical Engineering Dept.
Imp. Coll. of Sc. and Techn.
Exhibition Road
GB- London SW7 2AZ

Dr. H.M. ApSimon
Mechanical Engineering Dept.
Imp. Coll. of Sc. and Techn.
Exhibition Road
GB- London SW7 2AZ

Telephone number: (1) 589.51.11

Title of the research contract:

Pathways and systems pertaining to the urban environment.

List of projects:

1. Pathways and systems pertaining to the urban environment.

Title of the project no.: BI6-F-108-UK

Pathways and Systems Pertaining to the Urban Environment

Head(s) of project: Prof. A.J.H. Goddard Dr. H.M. ApSimon

Mechanical Engineering Department

Imperial College of Science Technology and Medicine

London SW7 2AZ UK

Scientific staff: Dr. R.J. Cannell Ms J. MacCurtain

I. Objectives of the project:

The first part of the study has been concerned with the impact of the highly variable nature of precipitation on reactor accident consequences. The importance of this has been demonstrated by the Chernobyl accident, which has been used to evaluate the methods developed. The second part of the study has been aimed at developing models for the protection of the individual inside dwellings, with particular reference to the ingress, deposition and other transport processes of radioactivity in particulate form. Lack of experimental data led to the development of new experimental techniques that are being exploited under contract BI6-F-228-UK.

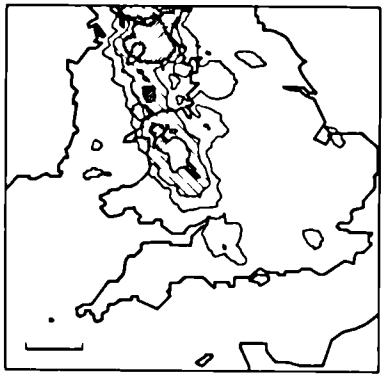
II. Objectives for the reporting period:

To carry out further work on the effect of physical and chemical processes on wet deposition, aided by other sources of support within the UK. In respect of particulate transport within dwellings, the development of a computer model to represent a range of occupant dose pathways within dwellings.

III. Progress achieved:

The first part of the project has now been completed. With the aim of providing good spatial and temporal resolution of rainfall an extensive data base of weather radar observations was established, and a computer model, RAINPATCH, developed to simulate the dispersal and wet deposition of a radioactive release to the atmosphere. Model and data base have been used in a study of 300 hypothetical releases, in which the deposition patterns over England and Wales were analysed.

The techniques developed within the contract were subsequently applied to produce a map of deposition of Cs-137/124 from Chernobyl over England and Wales. A continuation of the work financed by the NII and NERC in the UK



Wet deposition from the RAINPATCH model contour levels over $\geq$ 500 Bq m^{-2} (close-hatched), 1000 Bq m^{-2} (hatched) and 100 Bq m^{-2} (from Ref.1)

Measured Cs-137 on grass in Bq m^{-2} produced by the Institute for Terrestial Ecology

has illustrated how such deposition can be enhanced orographically by a factor several times larger than the increase in rainfall over high land. Variability in removal efficiency of different types of precipitation has also been explored.

In respect of the protection of individuals afforded by dwellings against the ingress and subsequent transport of radioactive aerosols, a computer model DHOMO has been developed. The processes represented in the model include infiltration of external air, deposition and resuspension indoors, and the mechanical ingress of contaminated material from external surfaces. In view of the significant lack of experimental data for such transfer

processes within dwellings, a programme of experimental measurements is in progress under the contract BI6-F-228-UK; these measurements have been conducted in part in collaboration with the Risø National Laboratory in Denmark.

IV. Objectives for the next reporting period:

The first objective of the contract has been completed. Research will continue on the second aspect of the study; in further refining the computer model for the protection afforded by dwellings against radioactivity in the atmosphere in particulate form, as experimental data are obtained.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Risø National Laboratory, Denmark, (J. Roed)

VI. Publications:

1. ApSimon, H.M. and Simms, K.L. The use of weather radar in assessing deosition of radioactivity from Chernobyl across England and Wales. Atmospheric Environment. 1988. 22. p. 1985-1900.

2. ApSimon, H.M., Simms, K.L. and Stott, P. The treatment of wet deposition in probabilistic accident consequence assessment. Proc. of CEC Meeting on PRA. 1988. Rome, January 1988.

3. ApSimon, H.M., Cannell, R.J., Goddard, A.J.H., McCurtain, J. and Mcguirk, J. The behaviour of aerosols within dwellings in assessing the consequence of the release of radioactivity to the environment. Proc.Int.Conf., Indoor and Ambient Air Quality, Imperial College, London, 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-106-B

Studiecentrum voor Kernenergie
SCK/CEN
Rue Charles Lemaire, 1
B-1160 Bruxelles

Head(s) of research team(s) [name(s) and address(es)]:

Ir. P. Govaerts
Dept.Stralingsbescherming
SCK/CEN
Boeretang, 200
B-2400 Mol

Telephone number: (014) 31.68.71

Title of the research contract:

Optimization of dose assessment models including the interface with environmental survey, for use in case of accidental releases.

List of projects:

1. Optimization of an emergency dose assessment and forecasting system.
2. Feasibility study of feedback of survey results.
3. Application of the dose assessment and forecasting model to generate reference scenarios for emergency response training.

Title of the project no.: 1

Optimisation of an emergency dose assessment and forecasting system.

Head(s) of project:
P. Govaerts

Scientific staff:
A. Sohier
J. Pauwels

I. Objectives of the project:

The project aims the extension of the existing dose assessment models at SCK/CEN, to be incorporated in a system allowing the execution of the two other projects, i.e. the feasibility study of the feedback of environmental measurements and the generation of scenarios for emergency response exercices. The dose assessment will consider all radionuclides and exposure pathways, relevant for decision making during the early emergency response phase, in case of a severe accident as considered by the current safety assessments.
The operationality of the model will be demonstrated.

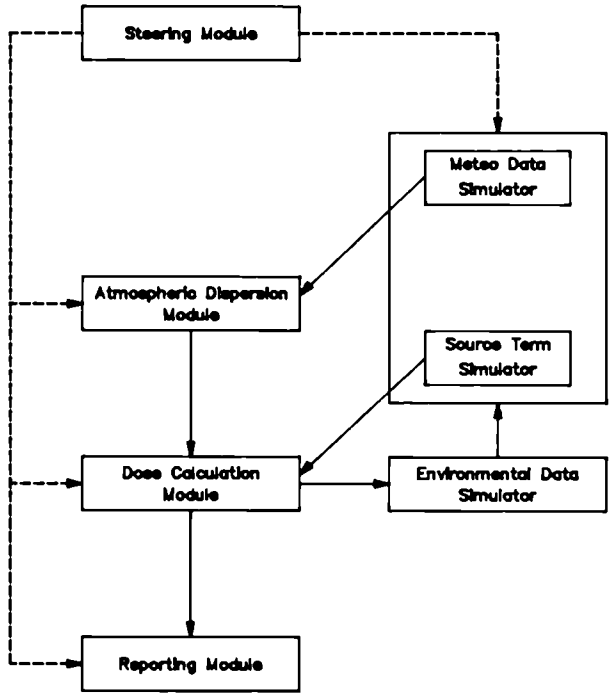
II. Objectives for the reporting period:

- Test of the source term simulator
- Coupling of existing modules to a more users friendly system.

III. Progress achieved:

1. METHODOLOGY

The project aims the development of a dose assessment model for use during accidental situations, as well as a tool to test and demonstrate the outcome of the two other projects (i.e. procedures to feedback environmental survey results to optimize model input parameters and development of emergency response scenarios). The general structure of the system is represented by the following figure.



2. RESULTS

- Quality assurance of the source term simulator

During earlier reporting periods a relatively simple model was developed to assess the reactor core inventory in function of power level, burn-up and cooling time. This model has been checked against a large number of runs by the ORIGEN-code, varying the above mentioned input parameters and the nature of the reactor fuel. Three typical cores were considered, i.e. : the large PWR-core, a fast-breeder core and a typical Material Testing Reactor (SCK/CEN BR2-reactor)-core. The comparison learned that for some radionuclides the simple model led to unacceptable differences. For this reason, the analytical model has been partly replaced by a numerical one, interpolating between the data calculated by the ORIGEN-code for a PWR-core.

This inventory can however hardly be considered as an acceptable approach for the other fuels. Real-time systems for installations with non-PWR reactors have to be fitted-out with a specific inventory calculation module.

- Quality assurance of the dose calculation module

The dose calculation module has been checked against results obtained by the SCK/CEN environmental dose calculation model - DOSDIM - accident. The comparison allowed the debugging of the real-time system.

3. DISCUSSION

The calculation model modules are developed and tested as foreseen.

IV. Objectives for the next reporting period:

- Optimisation of the user-system interaction.
- Optimisation of the reporting module.
- Documentation of the system by a descriptive report and a users'manual.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Title of the project no.: 2

Feasibility study of feedback of survey results.

Head(s) of project:
P. Govaerts

Scientific staff:
A. Sohier
J. Pauwels

I. Objectives of the project:

The project evaluates the feasibility of updating some input parameters to the dose assessment model by comparison of environmental survey results to the corresponding values predicted by the model.
In a first phase a system has to be developed allowing the testing of alternative procedures. In a second phase procedures will be established, tested and evaluated on their feasibility.

II. Objectives for the reporting period:

- Testing of the optimisation approaches, described by the 1987 progress report, in order to analyse the problems of an automatisation of a system, and the definition of a standard acceptance criterium.
- Simulation of examples of a realistic information flow with respect to the condition of the environment during an emergency.

III. Progress achieved:

1. METHODOLOGY

- Simulation of environmental survey results and selection of reported tracer experiments to test parameter optimisation techniques.
- Development of an optimisation technique for simple applications.
- Demonstration of this technique.

2. RESULTS

An extensive set of feedback exercices were performed on the basis of measured results during experimental tracer releases. The two methods, described in the progress report 1987 and presented at the CEC/OCDE seminar in Rome were tested.

The main problems can be summarized as follows :

1. The feedback exercice is only feasible as far as the monitoring data fit rather well the assumed model. The data sets used for the testing show however several cases where although the conditions are relatively simple, the bigaussian model doesn't fit at all.
2. The outcome of the optimisation can heavily depend on the choice of the acceptance criteria, as reported in the above mentioned publication.
3. The delay between the onset of the releases and the availability of a sufficient data set for reliable feedback applications might be unacceptable for accident-management purposes (see also project 3).

Two options to increase the reliability of the feedback procedure have been discussed and will be further analysed during the next reporting period ; i.e.

- the use of real-time environmental monitoring data. This approach asks to cope with fast concentration fluctuations. These might however be damped in case of external gamma-monitoring. We try to obtain a well-documented data base of short-term monitoring results (concentrations and dose rates with short averaging times) to continue this analysis.
- the introduction of a probability distribution to each monitoring result, indicating the generic quality of the model to fit with the actual measurements.

3. DISCUSSION

The project is progressing as proposed by the previous report. The simulation of information flow during an emergency is discussed by project 3.

IV. Objectives for the next reporting period:

- Documentation of feasibility tests.
- Definition of generic probability functions to be assigned to environmental monitoring data.
- Introduction of those distributions in the numerical feedback approach.
- Discussion of problems related with use of on-line monitors.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

P. Govaerts, A. Sohier : Feedback of Environmental Survey Data for the Optimisation of the Input Parameters of Assessment Models during an Emergency.
Joint OECD(NEA)/CEC Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome (Italy), January 1988.

P. Govaerts, A. Sohier : Optimisation of input parameters of assessment models during an emergency.
17th NATO/CCMS Int. Technical Meeting on air pollution modelling and its application, Cambridge (UK), September 1988.

Title of the project no.: 3

Application of the dose assessment and forecasting model to generate reference scenarios for emergency response training.

Head(s) of project:

P. Govaerts

Scientific staff:

A. Sohier
J. Pauwels

I. Objectives of the project:

The model developed by the first project will be used to generate a set of scenarios for the purpose of emergency response training. The scenarios will be selected according the multiple objectives an emergency exercice can have. The scenarios are represented by the schedule of information flows between the different functions of an emergency response team, and will discuss the decision making process.

II. Objectives for the reporting period:

- Further development of a computer assisted system for scenario selection based on the perceived needs for training.
- Development of a standard format for the representation of the scenarios.

III. Progress achieved:

1. METHODOLOGY

In order to obtain a set of emergency scenarios, which can be selected in a logical way for the training of specific tasks of emergency response, following tasks can be distinguished :

1. Collection of raw data base for scenario selection ;
2. Elaboration of a logical scheme for scenario selection ;
3. Definition of a limited number of scenarios classified according the objectives of the exercice ;
4. Standard description of the scenarios ;
5. Redaction of a detailed script for each scenario.

2. RESULTS

1. A set of 20 meteorological sequences, of 20 successive half-hour periods each, representing a broad range of atmospheric conditions has been selected, using real data of the high meteo-tower of Mol.
2. The system to define coarsely a source term and a meteorological situation, leading to a radiological situation intended by the exercice planned was further developed during the reporting period.
3. The delay of the information flow related to environmental monitoring was analysed, using typical procedures. A small basic-model was programmed to simulate the procedures, using realistic times for unit operations. Figure 1 shows a generic result in the form of availability of information in function of time, for several preplanned monitoring circuits.

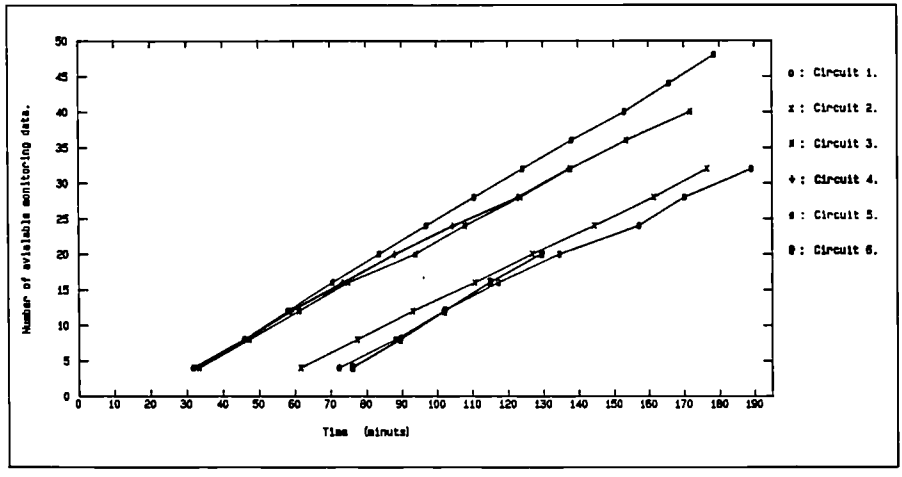


Fig. 1 : Typical simulation of the availability of monitoring data in function of time for several circuits

4. A general scheme of the emergency scenarios has been discussed. Exercices intend to check :
   - communications between units
   - efficiency of one or more units

   The function of a unit can be the transformation of information or the execution of an action. The scenario has to provide all input information for the unit to test. This input will be adapted to the response of the unit by feeding back the output of the unit. This feedback will only affect the availability of information to the players of the exercice. The basic structure of the organisation for which scenarios will be prepared is shown in by figure 2.

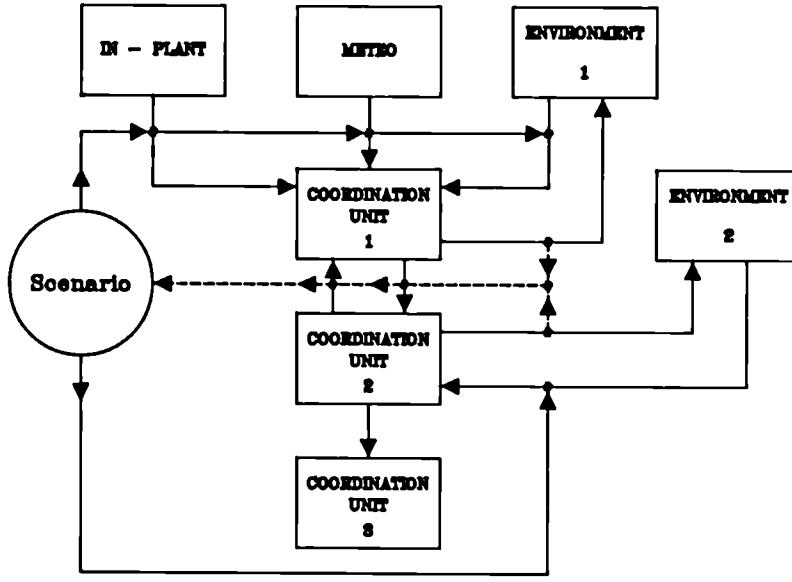


Fig 2. : Basic structure for emergency response exercise scenarios.

3. DISCUSSION

During the reporting period the main emphasis was still on the development of basic tools for the realistic simulation of accidents. The work on the actual format of the scenario-description has not yet started.

IV. Objectives for the next reporting period:

- Finalize the procedure for scenario definition.
- Documenting the procedures.
- Redaction of a set of scenarios.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
11 Charles II Street
GB- London SW1 4QP

Contract no.: BI6-F-131-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M.R. Hayns
Safety and Reliability Directorate
Wigshaw lane, Culcheth
GB- Warrington WA3 4NE

Telephone number: 31.244

Title of the research contract:

Evaluation and development of models used in assessing the consequences of accidental releases of radioactivity.

List of projects:

1. Evaluation and development of models used in assessing the consequences of accidental releases of radioactivity.

Title of the project no.:

Evaluation and development of models used in assessing the consequences of accidental releases of radioactivity.

Head(s) of project:

Dr B Y Underwood

Scientific staff:

Dr B Y Underwood

I. Objectives of the project:

To examine aspects of the simplified approaches conventionally used in consequence assessment from a more fundamental viewpoint, thereby allowing an appraisal of the range of applicability of the simple models and enabling the formulation of appropriate modifications where necessary.

II. Objectives for the reporting period:

To progress the work on the use of Lagrangian-particle techniques in investigating the range of applicability of simple models for including gravitational settling in a Probabilistic Consequence Assessment code.

To this end, to first work out how to include a realistic lower boundary condition into the Lagrangian-particle (Monte Carlo) technique, working initially with an idealised homogeneous boundary layer for conceptual simplicity but then moving on to the more realistic (vertically-) inhomogeneous case.

To compare the Monte Carlo model predictions first with experimental data and then with the predictions of the simpler models.

III. Progress achieved:

Methodology

Probabilistic Consequence Assessment (PCA) codes are required to handle a variety of accident scenarios in a wide range of potential weather conditions. Consequently, simple parameterised models of atmospheric dispersion-such as the Gaussian plume model - are usually employed, together with heuristic extensions to account for deposition and other processes. However, there is a need to check these model extensions against more rigorous approaches and against experimental data in order to ensure that the bias and uncertainty introduced by the simplification do not seriously impair the validity of the conclusions that can be drawn from the results.

Conventionally, deposition in dry conditions has been treated via the Source Depletion Approximation (SDA) which is usually adequate for micron-sized particles. However, it is now recognised that PCA codes should be capable of dealing with larger particles, which have an appreciable gravitational settling velocity. Little work has been done to date to compare extensions to the Gaussian plume model for dealing with larger particles, principally versions of the 'Tilted-Plume Approximation' (TPA), with the results of a more soundly-based theory (or experiment) to define their range of applicability and accuracy.

For the smaller particles, eddy-diffusivity theory can supply the more-detailed model against which the SDA is compared. However, the use of eddy-diffusivity theory to provide the 'benchmark' is called into question by the detailed understanding of transport processes in the atmospheric boundary-layer when the focus of interest is the first few kilometers downwind of a source elevated by tens of metres. Specifically, a particle tends to retain its current value of turbulent velocity for a finite time related to the size of the main energy-containing eddies in the flow and this is not represented in eddy-diffusivity theory. In the present work, a model is developed which allows for this finite 'memory': concentration fields are built up by tracking many individual particle histories as they experience a 'random walk' under the influence of the turbulence.

This model is first applied to non-depositing pollutant, and its predictions checked against experimental data for this case. The model

is then applied to settling particles and the predictions compared with measurements taken in a number of field trials. It is then used to evaluate the simple extensions to the Gaussian model used in assessment work.

Results

When applied to non-depositing pollutant, the model developed produces results in good agreement with experimental data for this case. When applied to settling particles, the model predictions give a better fit to experimental data than other available models, giving confidence in its ability to shed light on the performance of the simple Gaussian extensions. In comparisons with the latter, it is found that the SDA itself works well even for particles with appreciable gravitational settling velocity, up to the point at which the latter becomes about one half the turbulent velocity scale in the atmosphere, whereas for larger particles the tilted-plume approach works better.

However, even the TPA becomes inaccurate as the ratio of settling velocity to turbulent velocity (termed V_S) increases much beyond unity. In particular, it underpredicts the maximum ground-level concentration (but not by a large factor) and underestimates the rate of fall-off of the ground level concentration with distance beyond the location of its maximum; ie concentrations and deposit densities are overestimated at greater distances. For example, for $V_S = 2$ the 'best' variant of the TPA tested overestimates by a factor of 50% at the location where the reference-model concentration has fallen to 1/10 of its maximum and a factor of 4 where it has fallen to 1/000; the discrepancy grows rapidly with increasing V_S.

Discussion

The above results can be used in consequence-assessment codes to determine which of the two Gaussian extensions considered should be applied in given circumstances. If for the range of particle-sizes of interest the limitations of the TPA are found to have a major influence on the overall picture, the present work can provide the basis of an improved parameterisation.

IV. Objectives for the next reporting period:

The above-reported work has so far only treated the simplest case of particles dispersing in the neutral surface-stress layer of the atmosphere, for which the distribution of turbulent vertical velocities can be assumed uniform with height. A wider range of pertinent problems could be tackled if the basic model can be extended to deal with vertical non-uniformity of this distribution, for example the extension to other atmospheric stabilities and to the problem of deposition in canopies. The fundamental work required to treat the non-uniformity will be undertaken with the aim of tackling some of these applications.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Scientific Journals

UNDERWOOD B Y (1987). Dry deposition to an urban complex. Radiation Protection Dosimetry 21, 21-32.

Reports

UNDERWOOD B Y (1988). Deposition in foggy conditions. SRD R487. Safety and Reliability Directorate.

UNDERWOOD B Y (1988). Gravitational settling of particles dispersing from an elevated point source in the neutral surface layer of the atmosphere. Contract report to CEC.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Central Electricity Generating
Board, CEGB
Berkeley Nuclear Laboratories
Berkeley
GB- Glos. GL13 9PB

Contract no.: BI6-F-209-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. T. Healey
Berkeley Nuclear Laboratories
CEGB
Berkeley
GB- Glos. GL13 9PB

Telephone number: (453)81.04.51

Title of the research contract:

An analysis of uncertainties in inhalation and ingestion dose estimates arising from uncertainties in dosimetric and foodchain transfer data.

List of projects:

1. An analysis of uncertainties in inhalation and ingestion dose estimates arising from uncertainties in dosimetric and foodchain transfer data.

Title of the project no.:

An Analysis of Uncertainties in Inhalation and Ingestion Dose Estimates Arising from Uncertainties in Dosimetric and Foodchain Transfer Data

Head(s) of project:

Dr. S. Nair,
CEGB, Berkeley Nuclear Laboratories,
Berkeley,
Glos GL13 9PB

Scientific staff:

Dr. S. Nair
Mr. A.C. Ponting

I. Objectives of the project:

The primary objective is to determine the uncertainty in ingestion dose estimates arising from uncertainties in the foodchain transfer and in the internal (human) dosimetry.

The secondary objective is to estimate the sensitivity of these uncertainties to the statistical sampling method chosen.

II. Objectives for the reporting period:

First, develop a coupled foodchain/metabolic model for the exposure of the thyroid due to intake of I-131 in milk.

Second, perform an uncertainty analysis on the thyroid dose, identifying key contributors to the overall uncertainty.

III. Progress achieved:

Foodchain model description, seasonality studies and uncertainty analysis of activity concentrations

The results of this work were presented at the NEA/CEC Seminar on Recent Advances in Reactor Accident Consequence Assessment at Rome in January 1988 [1]. Calculations were made of the dependence of the transfer to a number of foods arising from discrete depositions of I-131, Cs-134 and Cs-137 occurring at different times of the year. The observed time variations were interpreted in terms of the dominant transfer processes and scenarios identified where the ingestion pathway is dominant.

Uncertainty analyses were also carried out for these scenarios, ignoring parameter correlations, in two different ways:

(i) every single combination of parameter values was run through the model and a complete probability distribution function constructed for selected nuclides and foods. The use of efficient numerical integrators for the crop sub-model and analytical solutions for the animal product sub-model was essential to the success of this exercise, and

(ii) the Latin Hypercube Sampling (LHS) technique [2] was used to reproduce the probability distribution functions for different numbers of trials.

Results from (i) and (ii) were compared to determine the optimum number of trials with the LHS technique.

Coupled foodchain/metabolic model and associated uncertainty analysis

The progress report for the previous year noted that an analytical expression had been derived for the I-131 ingestion dose-intake factor. This expression was subjected to an uncertainty analysis arising from uncertainties in the fractional uptake from blood to the thyroid, the thyroid mass and the effective half-life of I-131 in the thyroid. The uncertainties in the expected value for dose-intake factor derived from considering the 1st and 99th percentiles of the resulting probability distribution were found to be about a geometric factor of 3 around an expectation value of 3.5E-7 Sv/Bq. This compares with the ICRP30 single point value of 4.8E-7 Sv/Bq.

The metabolic model was coupled with the model for time-dependent uptake into milk and the combined foodchain/metabolic model was also subjected to an uncertainty analysis. The results indicate an uncertainty of $x5/\div 10$ on the expectation value (based on the 1st and 99th percentiles), this being in turn about a factor of two smaller than the single point "best estimate" value.

A comparison was also made between the results of uncertainty analyses carried out using the technique developed earlier at CEGB [3] and different numbers of trials using the Sandia Latin Hypercube scheme. The results indicated that the Sandia scheme predicted the 1st and 99th percentiles to within a few percent for runs using 100 trials.

Work currently in hand has extended the coupled foodchain/metabolic models to include radiocaesium. These, and the results of the associated uncertainty analyses, will be described in a final CEGB report to be produced in 1989.

[1] S. Nair, A.C. Ponting, 1988, "Seasonality and uncertainty aspects of ingestion dose: Some preliminary results", Proc. of a NEA/CEC Seminar on Recent Advances in Reactor Accident Consequence Assessment, Rome, January 1988.

[2] R.L. Iman, M.J. Shortencarier, 1984, "A FORTRAN77 program and user's guide for the generation of latin hypercube and random samples for use with computer models", Report NUREG/CR-3624 (SAND83-2365).

[3] S. Nair, A.C. Ponting, 1985, "An analysis of uncertainty and of dependence on season of year of ingestion population dose arising from design basis accidents in advanced thermal reactors", CEGB report TPRD/B/0628/N85.

IV. Objectives for the next reporting period:

(i) Complete the development of coupled foodchain/metabolic models for radiocaesium ingestion in milk and in green vegetables.

(ii) Perform uncertainty analyses on the average whole body dose from radiocaesium ingestion, identifying the key contributors.

(iii) Produce final contract report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Contact was maintained with Studsvik on their PRISM method for carrying out uncertainty analyses. This extended an existing collaboration involving a joint analysis of Chernobyl data.

VI. Publications:

[1] S. Nair, A.C. Ponting, 1988, "Seasonality and uncertainty aspects of ingestion dose: Some preliminary results", Proc. of a NEA/CEC Seminar on Recent Advances in Reactor Accident Consequence Assessment, Rome, January 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Institut National de la Santé et
de la Recherche Médicale, INSERM
101 rue de Tolbiac
F-75654 Paris cedex 13

Contract no.: BI6-F-126-F

Head(s) of research team(s) [name(s) and address(es)]:

M. D. Hémon
U. 170
INSERM
16 avenue Paul Vaillant Couturier
F-94807 Villejuif

Telephone number: 45.59.50.30

Title of the research contract:

Statistical methods for the analysis of geographical correlations, application to the analysis of the correlation between population radiation exposure and cancer mortality.

List of projects:

1. Statistical methods for the analysis of geographical correlations, application to the analysis of the correlation between population radiation exposure and cancer mortality

Title of the project no.: B16-F-126-F

Statistical methods for the analysis of geographical correlations, application to the analysis of the correlation between population radiation exposure and cancer mortality.

Head(s) of project:

Dr. S. RICHARDSON

Scientific staff:

Dr. D. HEMON, Dr S. RICHARDSON

I. Objectives of the project:

The research project presented here has a double purpose : first to investigate statistical methods suited to the analysis of models of association between spatially defined variables, then to apply these methods to the study of the joint variations of risks factors such as low dose radiation or industrial pollution together with some health indicators such as mortality for cancer of specific sites.

II. Objectives for the reporting period:

. Extend the tests of association developped to the testing of partial correlations in order to be able to adjust for the effect of confounding variables when studying the relationship between cancer mortality and low doses radiation.

. Investigate the significance level and the power of these tests.

. Carry out the implementation of these tests on French geographical data.

III. Progress achieved:

In the last three reports we have described modified test of association for spatially autocorrelated variables X and Y and simulation models designed to evaluate the performance (type I errors and power) of the modified test and compare them to existing procedure. In this report we describe an extension of the test of the correlation to the case of several variables and give results showing satisfactory type I errors and power of this method.

1. Methodology

The modified test of correlation which was developped is based on a modification $\hat{M}$ of the degrees of freedom (d.o.f.) of the test of the correlation coefficient r_{XY} between pairs of observations (X_α, Y_α), $\alpha \in A$, where A is a set of N locations. $\hat{M}$ is defined by $\hat{M}=1+\hat{\sigma}_r^{-2}$ where $\hat{\sigma}_r^2$ is an estimation of the variance of r_{XY} which takes into account the spatial autocorrelation of both X and Y. The modified t-test of r_{XY}, $t_{\hat{M}-2}$ rejects the null hypothesis of no association when $| (\hat{M}-2)^{1/2} \; r(1 - r^2)^{-1/2} |$ is larger than the critical value of the t-distribution with $\hat{M}-2$ d.o.f.

We now describe an extension of this method for testing the association between two variables (Y_α, Z_α) adjusted on a third one, X_α, $\alpha \in A$. Its generalisation to any number of adjustment variables is straightforward.

We suppose that the 3N vector $\begin{pmatrix} X \\ Y \\ Z \end{pmatrix}$ follows a multivariate normal distribution.

Then the joint distribution of (Y,Z) conditionnal on X is also a multivariate normal. We can therefore test the hypothesis : $\rho_{YZ.X} = 0$, where $\rho_{ZY.X}$ is the partial correlation between Y and Z conditional on X, by testing that the correlation between the residuals of the regression of Y on X and of Z in X is zero. Hence, the method previously outlined can be extended to test the partial correlation.

In practice, this implies using the modified $t_{\hat{M}-2}$ statistic on the residuals of the linear regression of Y on X and of Z on X respectively. These residuals need therefore to be estimated. We are proposing to do this by ordinary least squares (O.L.S.), thus ignoring the autocorrelation, since the O.L.S regression estimates are unbiased.

In summary the following steps are followed :

1 - regress Y on X by O.L.S giving estimated residuals $\hat{U}$

2 - regress Z on X by O.L.S giving estimated residuals $\hat{V}$

3 - test the correlation coefficient between $\hat{U}$ and $\hat{V}$ using the modified test statistic $t_{\hat{M}-2}$ given with $\hat{M} = (\text{var } r_{\hat{U},\hat{V}})^{-1} + 1$

In order to check whether the estimation of the residuals by O.L.S could influence the performance of the test, a Monte-Carlo simulation was carried out to check the type I errors and to give some indication on the power of the proposed method. The simulation was carried out on an irregular grid of points defined by the administrative centers of the French *département* (N = 82). Spatial dependence was introduced directly on the variance-covariance matrix of the multivariate normal distributions by considering a disc model with autocorrelation parameter $\rho(1)$ (see preceeding reports). A 5 % nominal level was set for the modified $t_{\hat{M}-2}$ test.

2. Results

a) Performance of the modified test of partial correlation

Results on type I errors are shown in Table 1.

The performance of the modified $t_{\hat{M}-2}$ statistic is satisfactory as the value 5 % belongs to all the confidence intervals and there is no systematic variation with increasing autocorrelation.

As an illustration, the empirical variance of $r_{YZ.X}$ is also shown and one can see that it increases significantly with the autocorrelation $\rho(1)$. As expected, the type I errors of the non adjusted standard test of partial autocorrelation are greatly inflated for high values of $\rho(1)$.

The power of the modified test was then investigated for value of $\rho_{YZ.X}$ equal to 0.2 and 0.4 and a 5 % nominal significance level. Higher values of $\rho_{YZ.X}$ led to a power close to 100 % and were less informative.

In order to have a reference value for the power the modified $t_{\hat{M}-2}$ statistic, equivalent sample size, N* :

$$N^* = 2 + (1 - \rho^2_{YZ.X})^2 / v_e$$

was also calculated. This number N* was used to compute the reference value π, power of the standard test of $\rho_{YZ.X}$ based on N* observations.

A summary of the results is given in Table 2. Clearly the test $t_{\hat{M}-2}$ perform satisfactorily as its power is comparable to π.

b) Implementation of these tests to French geographical data

Computer programms were developped which carried out this testing procedure on French data collected by départements. The testing method was applied to study the association between lung cancer and industrial factors after adjustments on cigarette consumption. Interesting results came to light on significant links with the metal industry, general engineering and mining which agreed with results of individual epidemiological studies. This confirmed the suitability of the methods developped for studying problems in geographical epidemiology.

3. Discussion

In this report, a test of partial correlation between spatially defined variables has been described and shown to have a satisfactory performance. This test is needed to be able to take into account confounding variables when studying the relationship between cancer mortality and low dose radiation. It would be interesting to compare on some examples the results given by this test to those of a multiple regression with spatially parametrised error variance-covariance matrix.

In the last part of the project, the data file both on mortality and on indices of low dose radiation will be updated and a detailed analysis using the statistical techniques developped will be carried out.

Table 1

Type I errors (per cent) of the $t_{\hat{M}-2}$ statistic for testing the partial correlation coefficient $r_{YZ.X}$ between $Z = aX + U$ and $Y = cX + W$ after adjusting on X.
500 simulations are carried out for several levels of autocorrelation in X,U and W ($\rho_X(1) = \rho_U(1) = \rho_W(1)$), with $a = 2c = 0.204$. As reference, the empirical variance of $r_{YZ.X}$ and the type I errors (per cent) for the classical test of $r_{YZ.X}$ based on N = 82 observations are also indicated.

autocorrelation $\rho(1)$ for variables X,U and W	0.0	0.2	0.5	0.8
empirical variance of $r_{YZ.X}$	0.0117	0.0129	0.0171	0.0500
% type I errors for $t_{\hat{M}-2}$ (95 % CI)	4.2 % [2.4 %-6.0%]	3.8 % [2.1 % - 5.5%]	4.8 % [2.9 % - 6.7%]	3.8 % [2.1 % - 5.5%]
% type I error for the t test * of $r_{YZ.X}$ based on N observations (95 % CI)	5 % [3.1 %-6.9%]	5.6 % [3.6 % - 7.6%]	10.6 % [7.9 % - 13.3%]	38.6 % [34.3%-42.9%]

* test of $\sqrt{N-3}\ r_{YZ.X} / (1 - r^2_{YZ.X})^{1/2}$ as a t_{N-3} distribution

Table 2

Power of the modified $t_{\hat{M}-2}$ statistic for testing the partial correlation coefficient $r_{YZ.X}$ between $Z = aX + U$ and $Y = cX + dZ + W$ after adjusting on X.
500 simulations are carried out for several levels of autocorrelation in X,U and W ($\rho_X(1) = \rho_U(1) = \rho_W(1)$), with $a = 2c = 0.204$. N* is an equivalent sample size base on v_e, the observed variance of $r_{YZ.X}$ and π is the power of a standard test of $r_{YZ.X}$ based on N* observations.

$\rho_{YZ.X}$	$\rho(1)$	0.0	0.2	0.5	0.8
0.2	v_e	0.0111	0.0121	0.0156	0.0465
	N*	85	78	61	22
	π	44. 8 %	40.4 %	33.3 %	10.8 %
	power of $t_{\hat{M}-2}$	43.4 %	41.7 %	34.6 %	13.6 %
0.4	v_e	0.0087	0.0094	0.0120	0.0372
	N*	83	77	61	21
	π	96.4 %	95 %	89.1 %	41.7 %
	power of $t_{\hat{M}-2}$	96 %	95.4 %	91 %	46.8 %

IV. Objectives for the next reporting period:

. Investigate the feasability of defining non parametric permutation tests of association where the permutations would preserve some aspect of the spatial structure.
. Compare the results of the modified tests to those of a multiple regression with spatially parametrised variance-covariance matrix of errors.
- update the mortality data file and collect available geographical data on background radiation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. P. Clifford, Mathematics Institute, University of Oxford.

VI. Publications:

Clifford P., Richardson S., Hémon D. Assessing the significance of the correlation between two spatial processes, accepted for publication in Biometrics (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-110-UK

Head(s) of research team(s) [name(s) and address(es)]:

Ms. M.D. Hill
Assessments Department
NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: (235) 83.16.00

Title of the research contract:

Establishment of authorized limits for effluent releases and implementation of the ALARA principle.

List of projects:

1. Issues in establishing authorized limits for effluent releases.
2. Methods for the practical implementation of the ALARA principle.

Title of the project no.: 1

Issues in establishing authorised limits for effluent releases

Head(s) of project: M D Hill

Scientific staff: S M Haywood, C A Robinson, N P McColl

I. Objectives of the project:

The objectives of this project are to explore a number of radiological protection issues which have implications for the establishment of authorised limits for effluent releases, and hence to provide results which will be useful to the authorities responsible for setting these limits. The issues to be considered include: methods for defining critical groups, source upper bounds, the application of dose limits, and comparisons between discharge of effluents and trapping, immobilisation and disposal of radionuclides in solid form.

II. Objectives for the reporting period:

Further consideration would be given to theoretical aspects of critical group philosophy and there would be a more detailed analysis of available distributions of dietary intake data. It was proposed to use available statistical techniques to investigate the distribution of dose in a population by considering the distributions of factors which affect the dose received, such as habits (e.g. dietary intake), dose per unit intake and concentrations of radionuclides in environmental materials, such as foodstuffs.

III. Progress achieved:

A necessary input to this study is data on the variation between individuals in the consumption of important foodstuffs, and in dose per unit intake for several radionuclides following ingestion.

A certain amount of information is already available on the variation in consumption rates of several key foodstuffs, but further review of the information available in this area has indicated several studies currently in progress in the UK which should yield valuable data for this study. This work is being carried out at the Ministry of Agriculture, Fisheries and Food (MAFF). We are in contact with the researchers involved in the MAFF studies and have indicated to them our data requirements, with the result that additional data is now being obtained. This data will be available to us later in 1989.

Data on the variation between individuals in the dose they receive following intake has been reviewed by staff in the Biomedical Effects Department of NRPB. Probability distributions for doses per unit intake for several key radionuclides have been obtained. Work is continuing for several additional radionuclides.

Because of the delay in obtaining data on variation in dose per unit intake, and the additional data which will shortly become available on the variation in intake rates of foodstuffs, further progress has not been made on this study in 1988.

Since there has been an enforced delay in carrying out the work originally proposed for this year, work originally planned for 1989 has been brought forward. This work is an investigation of existing systems of radioactive discharge and critical group dose limitation around the world. Systems applied in EC countries have recently been reviewed by the CEC[1]. Under the present contract a compilation of data on national policies on discharge limitation and critical group dose estimates for non-EC countries has been started.

Reference

1. Commission of the European Communities. Methods for fixing discharge limits for radioactive effluents from nuclear installations in the member states: a review and analysis. Radiation protection number 42. Luxembourg, November 1988.

IV. Objectives for the next reporting period:

When the new data on the variation in intake rates of foodstuffs becomes available, the work described above will continue. Following determination of the important factors influencing critical group doses, a more quantitative approach to the identification and definition of a critical group will be developed. The investigation of systems of discharge limitation will be continued. These systems will be compared with current proposals and recommendations of international bodies. In addition the relative importance in terms of public exposure, of factors such as local habits, type of operation, and the system of discharge limitation will be investigated.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Title of the project no.: 2

Methods for the practical implementation of the ALARA principle

Head(s) of project: A D Wrixon

Scientific staff: J R Croft, A P Hudson, P Saunders

I. Objectives of the project:

(i) To review the difficulties which have arisen in the practical implementation of ALARA;

(ii) to suggest methods by which these difficulties may be resolved;

(iii) to develop a simple general framework for future ALARA studies; and

(iv) to demonstrate the use of this framework by applying it in example studies.

II. Objectives for the reporting period:

The objectives for 1988 were to consolidate the work of the previous years and to prepare a draft of the final report.

III. Progress achieved:

The early part of the project concentrated on two aspects; the development of a structured approach to the practical implementation of the ALARA principle, known as the ALARA Procedure, and case studies involving the application of this approach. This work included a review of the appropriate decision-aiding techniques, cost-effectiveness analysis, cost-benefit analysis, multi attribute utility analysis and multi criteria outranking analysis. More recently effort has been focusing on how the ALARA principle can be implemented in the operational situation through the use of ALARA Audits and Predictive ALARA Plans. During 1988, a number of papers were prepared which present the results of these considerations and raise some of the areas requiring further study, one of the most important being the collection of relevant dosimetric data[1,2,5,6]. The outline structure of the project report produced in 1987 has been modified to reflect the more recent work, the modified structure being shown on the attached sheets. Complete drafts of the first two sections were prepared during the year and substantial progress has been made in determining the content of the final section.

During 1988 the CEC Third European Scientific Seminar on Radiation Protection Optimisation took place in Madrid and provided a focus for the presentation of the work carried out under this project[5,6,7]. Project staff from NRPB and CEPN were heavily involved in organising the Seminar and were responsible for 30% of the papers.

IV. Objectives for the next reporting period:

The aim for 1989 is to carry out the necessary redrafting work to produce the final report. This will take into account the papers and the discussion of the CEC's Third European Scientific Seminar on Radiation Protection Optimisation - Advances in Practical Implementation, 12-14 September, Madrid, and any comments received on the draft versions of the report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

CEPN
Boite Postal No 48
F-92280 Fontenay-aux-Roses
France

VI. Publications:

1. Croft J R and Lochard J, Status of achievements reached in applying optimisation of protection in design and normal operation of reactors. The Application of Optimisation of Protection in Regulation and Practice, Proceedings of an NEA meeting 16-18 March 1988, Paris.

2. Lochard J and Croft J R, Key Issues in the implementation of ALARA in operations, Journal of Radiological Protection, Vol. 8(2), 1988.

3.* Croft J R, Hudson, A P and Wrixon A D, Experience with ALARA Audits in the non-nuclear power industry.

4.* Croft J R, et al, Use of Cost-benefit analysis in the field of industrial radiography.

5.* Saunders P, et al, Methods for the practical implementation of the ALARA principle: the CEPN, NRPB Joint Project.

6.* Croft J R, ALARA in industry.

7.* Croft J R and Wall B, Review of ALARA in Medical Applications.

*Both Presented at the IRPA 7 Conference, 10-17 April 1988, Sydney.
*Presented at 3rd CEC Seminar on Radiation Protection Optimisation - Advances in Practical Implementation : 12-14 September 1988, Madrid.

CONTENTS

7. Other relevant factors
   - 7.1 Non-radiological risks
   - 7.2 Non-health detriment
   - 7.3 Social factors
     - 7.3.1 Integration of ethical and social factors in the ALARA process
       - (i) Equity in the protection of the public and the workforce
       - (ii) The perception of social risks
       - (iii) Future risks

8. Data collection
   - 8.1 Dose modelling
   - 8.2 Task-specific dosimetry
     - 8.2.1 Protection costs

9. Evaluation of α and β
   - 9.1 The value of α
   - 9.2 The value of β
     - 9.2.1 Willingness to pay
     - 9.2.2 Implicit values from previous decisions
     - 9.2.3 Costing against manpower requirements

10. Decision-aiding techniques
    - 10.1 Cost-effectiveness analysis
      - 10.1.1 Differential cost-effectiveness analysis
    - 10.2 Cost-benefit analysis
      - 10.2.1 Basic formulation of cost-benefit analysis
      - 10.2.2 Extended cost-benefit analysis
    - 10.3 Multi-attribute utility analysis (decision analysis)
      - 10.3.1 The cost of implementing the protection option
      - 10.3.2 The occupational collective dose associated with Option i
      - 10.3.3 The maximum individual doses
      - 10.3.4 The discomfort associated with the ventilation rates
    - 10.4 Multi-criteria outranking analysis
    - 10.5 The choice of the appropriate decision-aiding technique

11. Sensitivity analysis
    - 11.1 Factors and options
    - 11.2 Models and database
    - 11.3 Value judgements

SECTION III - IMPLEMENTING ALARA IN RADIATION PROTECTION PROGRAMME

12. Implementing ALARA in design and operations
    - 12.1 Introduction
    - 12.2 Commitment to ALARA
    - 12.3 Management systems
        - 12.3.1 ALARA Audits
            - 12.3.1.1 Principle
            - 12.3.1.2 Techniques
            - 12.3.1.3 Examples
        - 12.3.2 ALARA Predictive plan
            - 12.3.2.1 Principles
            - 12.3.2.2 Predictive plans in Design
            - 12.3.2.3 Predictive plans in Operation
    - 12.4 ALARA Data Base
    - 12.5 ALARA Criteria
        - 12.5.1 Reference levels
        - 12.5.2 Performance indicators

13. Implementing ALARA in strategic decisions

Annexes: Case studies

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-127-UK

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: (235) 83.16.00

Title of the research contract:

Methodology for evaluating the radiological consequence of radioactive effluents released in accidents.

List of projects:

1. Atmospheric dispersion and deposition in off-site accident consequence modelling.
2. The assessment of exposure due to deposited material.
3. Countermeasures to reduce the impact of accidental releases of radioactive material.
4. Uncertainty analysis.

Title of the project no.: 1

Atmospheric dispersion and deposition in off-site accident consequence modelling

Head(s) of project: M D Hill

Scientific staff: J A Jones, J A Williams

I. Objectives of the project:

The overall objective of the project is to develop improved and more comprehensive models for predicting rates and patterns of dispersion and deposition following accidental releases of radionuclides to atmosphere. These models have to be capable of being included in probabilistic accident consequence assessment (ACA) codes. The works forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The work in this reporting period centred on the need to make final decisions on the types of models which should be included in the MARIA code, and to plan the detailed code specification and description.

III. Progress achieved:

Progress has been made on four topics under this project: planning the overall structure of the MARIA computer program (to be called COSYMA); the program for meteorological sampling; a revised model for cloud gamma dose calculations; and studies of the importance of modelling deposition on skin.

The overall structure of the COSYMA package has been agreed with Kernforschungszentrum Karlsruhe (KfK). The package will be based on the KfK program UFOMOD extended to include features of the NRPB program MARC which are not in UFOMOD. COSYMA will include 5 different dispersion models which are appropriate for different situations. These include a Gaussian model with linear trajectories based on ADMARC, models with non-linear trajectories based on atmospheric conditions at a single site or derived from several meteorological sites and appropriate for dispersion over short or long ranges, and a model for long duration releases. The models to be included in COSYMA for other aspects of the analysis are described in the progress reports for the other projects.

The meteorological sampling module of MARC is being extended for inclusion in the COSYMA code. The current version of the code has been translated into standard Fortran-77 to enable it to be used on any computer. The modifications required to extend the program for application with a trajectory dispersion model, so that the sampling scheme includes the wind direction, are being considered.

A revised method of calculating cloud gamma doses has been derived. MARC uses three different approximations depending on the size of the plume. There are small discontinuities in the variation of dose with distance at the boundaries between the regions in which the different models are applied. The revised model is based on a different way of evaluating the integral of dose over the finite cloud which can be used for all the situations considered in MARC. The revised method is more accurate than any of the previous approximations and does not lead to discontinuities in the variation of dose with distance.

Recent work suggests that the shielding factor for deposited gamma dose for people indoors is 0.1 rather than the value of 0.5 used in earlier studies. This implies that deposited gamma dose is now a less important contributor to dose received shortly after an

accident, and hence to early deaths from an accident. Other work suggests that the LD50 for skin damage is lower than the value assumed in the current version of MARC, and hence that this could become a more important contributor to early death after an accident. There is, however, very little information on the deposition of material to skin compared with that to other surfaces. Scoping calculations suggest that death from skin damage could range from being a trivial contributor to being the dominant contributor to early death for plausible variations in the parameter values describing deposition to skin.

IV. Objectives for the next reporting period:

The main objective for the next reporting period is to finalise the COSYMA program system. A secondary objective to to carry out a series of sensitivity runs to enable advice to be given on the choice of model or parameter value for running the program in a range of conditions, including running it on small computers.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Kernforschungszentrum Karlsruhe GmbH

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VI. Publications:

Jones, J A, Williams, J A and Hill, M D. The importance of trajectory modelling in accident consequence assessments. In Proc. Joint CEC/OECD (NEA) Workshop on recent advances in reactor accident consequence assessment, Rome. January 1988, CEC EUR 11408 (1988).

Title of the project no.: 2

Assessment of exposure due to deposited material

Head(s) of project: M D Hill

Scientific staff: J R Simmonds, J Brown, A Walmsley, S M Haywood

I. Objectives of the project:

The aim of this project is to develop or improve models for predicting doses to people following deposition of radioactive material after an accidental release of radioactive material to atmosphere. The exposure pathways considered include: external irradiation from material deposited on the ground and on buildings, transfer of radionuclides through terrestrial foodchains, radionuclide transfer in freshwater bodies and in the marine environment, and inhalation of resuspended material. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The work in the last reporting period was centred on the need to decide what models should be included in the MARIA code, and to plan the specification and outline of the code.

III. Progress achieved:

1. External exposure resulting from urban contamination

The computer model to predict external doses from gamma-emitting material deposited in the urban environment, EXPURT has been used to identify which decontamination techniques are likely to be effective in reducing external gamma doses. The study has shown that the removal of impermeable surfaces, i.e. roofs, walls, interior surfaces and paved surfaces has a range of effectiveness in reducing per caput doses and dose rates but is, on the whole, not very effective. One of the more effective methods of reducing doses is the complete removal of walls which leads to a maximum reduction in integrated dose of about 50%, although the 'best estimate' of reduction is about 15%. Other decontamination techniques which may be employed such as steam cleaning and scrubbing, which are not as effective in removing contamination as surface removal, will lead to a smaller reduction in dose. The removal of soil or ploughing of soil appear to be more effective decontamination techniques, especially soil removal. However, soil removal is likely to be expensive both in terms of carrying out the decontamination and the disposal of the soil. Ploughing of soil to a depth of 15 cm in an urban area can remove up to 55% of the dose and can probably be termed a relatively 'cheap' option and one that is practicable.

For routine use of the EXPURT model a set of default values for the input parameters of the model are required. There parameter values should be so called 'best estimate' values and the most appropriate for routine running of the model. Following a preliminary sensitivity analysis carried out using the model, further revieving of literature was carried out for those parameters contributing most to the uncertainty in the predicted per caput doses and dose rates. Data and information available since the Chernobyl reactor accident in 1986 have been considered and there has been collaboration with other MARIA contractors who have been involved in extensive work in this area, particularly since the Chernobyl accident.

2. The transfer of radionuclides through terrestrial foodchains

Work is in progress on producing an EC terrestrial food distribution data base for use in probabilistic accident consequence codes. This data base will contain information on the regional exchanges of important

foodstuffs (e.g. milk, grain and green vegetables) to account for the flow of food from areas of production to areas of consumption. These data are needed to calculate the levels of individual doses arising as a result of consuming contaminated foods.

Data on food distribution outside the UK will be based on records of food imports and exports between Member States. Distribution in the UK is, however, being considered in more detail, taking into account internal distribution patterns. To obtain these distribution patterns, information has been sought from various organisations and food producers in the UK as well as from the published literature. For foods where such data are not available, the distribution pattern has been inferred from knowledge of the regional levels of production and consumption.

Work on the foodchain models, FARMLAND has continued under contract to the CEC within the programme on 'Underlying Data for Derived Emergency Reference Levels'. This work has included the comparison of the predictions of the FARMLAND models with measurement data collected in the European Community after the Chernobyl accident, the collection of information on agricultural practices and how these vary within the UK and Ireland and the comparison of the predictions of the models of GSF, Munich (ECOSYS) and FARMLAND. This work is being carried out with close collaboration with GSF and will form an input into MARIA, where information on variations in agricultural practice and model parameter values used in the models across the EC can be utilised. The FARMLAND models will be made available as part of the MARIA package with supporting documentation.

IV. Objectives for the next reporting period:

The work in the next reporting period will centre on the need to finalise the models for the MARIA package. Supporting work will be completed on the collection of relevant data for use with the models, e.g. information on the variation in agricultural practices within the European Community for use with the FARMLAND models, and documentation of the models.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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Postfach 3640
D-7500 Karlsruhe 1
Bundersrepublik Duetschland

VI. Publications:

Crick, M J, Brown, J, Hussain, Z and Walmsley, A. Identification of important parameters in urban dose assessment. Radiation Protection Dosimetry 21 (1-3) pp 181-188, 1987.

Brown, J, Crick, M J and Hill, M D. The Effectiveness of various decontamination techniques for reducing external radiation doses to people living in an urban environment. IN Proc. Joint CEC/OECD(NEA) Workshop on recent advances in reactor accident consequence assessment, Rome. January 1988, CEC EUR 11408, 1988.

Title of the project no.: 3

Countermeasures to reduce the impact of accidental releases of radioactive material

Head(s) of project: M D Hill

Scientific staff: S M Haywood, J R Simmonds, C A Robinson

I. Objectives of the project:

The objective of this work is to enable improved representations of the effects of countermeasures to be included in ACA codes, and thus to allow these codes to be used to provide a greater input into emergency response planning. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The main objective of the work in this reporting period was concerned with the planning and design of the MARIA code, and the development of the models to be included in it.

III. Progress achieved:

Work in this period has centred around the continuing development of an improved model for calculating the economic consequences of accidents, and preliminary plans for the incorporation of this model into the COSYMA code. Work in this project is being undertaken at both NRPB and KfK, and there have been a number of meetings through 1988 at which collaborative aspects of the study have been discussed. The work described in this progress report is limited to the parts of the study that have been undertaken at NRPB.

Significant progress has been made on the development of a model to predict economic consequences in the UK of accidental releases of radioactive material. Much of the necessary input data has either been collected or the source of this data determined. Although some additional data may be needed if the model is to be applied to calculate economic consequences in parts of the EC other than the UK, it is thought that the model itself is applicable to the EC in general. A draft report on the model and its data has been prepared. Throughout this year's work, Dr C Heady of University College, London has acted as a consultant to NRPB.

The model that has been developed calculates the costs of countermeasures (including evacuation, relocation, food restrictions and decontamination) and also of health effects in the exposed population. The treatment of the economic impact of evacuation and relocation in the model includes the cost of transport, accommodation and the costs arising from the inability of the moved population to work. Also included are the costs of the longer term loss of utility of the land and its assets, and the depreciation of these in value. For these longer term costs the ability to discount the costs occurring, to present day value, is included. The basic economic quantity used in the calculation of loss of income and capital costs is Gross Domestic Product (GDP).

In calculating the economic impact of food bans the model includes the cost of the lost production on the economy (including the cost of replacement supplies), the cost of disposal of the food, and the lost capital value of the affected land. Again, the ability to discount the longer term costs, to present day value, is included. Consideration is currently being given to sources of data on the costs of disposing of contaminated milk, livestock and crops.

A detailed model for the calculation of the costs of decontamination of land, buildings, etc. is currently being developed. This model will include the cost of a number of levels of decontamination for several types of land surface. Aspects included are the cost of the cleaning process in terms of equipment, materials, disposal, transport and labour.

The costs included in the model for calculating the economic impact of health efects include the costs of medical treatment and the individual's production potential. These may be regarded as 'direct' costs, i.e. those which have a directly measurable effect on the economy. The model also includes the capacity to measure non-pecuniary costs related to the theoretical valuation of life. Consideration is currently being given to sources of data on the costs of medical treatment of radiation induced effects.

There has been a review of available information on land-use data in the UK, including the possible use of satellite information and aerial photography, which has concluded that the data currently available for the UK as a whole is of poor quality and also out-of-date. The implication of this is that land-use related methods of assessing economic impact will not be appropriate for the majority of the UK until improved data are available.

Consideration has been given to the incorporation of this model into the COSYMA code, and the coding and interfaces that will be required. A preliminary note on the structure of this module has been prepared at NRPB, for discussion purposes. In the note, consideration is given to the form of the output of the module, and also to the input that is required from other parts of the COSYMA code. Preliminary discussions with KfK have indicated that the majority of the required input will be obtainable from the code. The note also considers the internal structure of the module and the form of the various subroutines required.

IV. Objectives for the next reporting period:

The work in the next reporting period will centre on the need to finalise the models for the COSYMA package. The coding required to implement in the COSYMA code the model described above, will be constructed jointly with staff at KfK, together with the addition of any features necessary to assess economic consequences in the FRG, and more broadly in the rest of the EC. The remainder of the data required by the model will be collected. Once coding is completed the module will be tested and example applications produced.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

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Bundersrepublik Deutschland

VI. Publications:

1. Hill, M D. Methods for using the results of probabilistic accident consequence assessments in decisions on the siting, design and operation, of nuclear installations. IN proceedings of the joint CEC/OECD(NEA) workshop on recent advances in reactor accident consequence assessment, Rome, January 1988. Report EUR 11408 (1988).

Title of the project no.: 4

Uncertainty analysis

Head(s) of project: M D Hill

Scientific staff: J R Simmonds, J A Jones, S M Haywood

I. Objectives of the project:

The aim of the project is, on the basis of applying various techniques available for uncertainty and sensitivity analysis of large computer models, to select the techniques which are most appropriate for analysing the uncertainty in probabilistic risk assessments. The techniques will then be used to identify the major contributors to uncertainty in such assessments. The work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The main objective of the work in this reporting period was concerned with the planning and design of the MARIA code. The aim was to design the code in such a way that uncertainty analyses could be easily performed. An analysis of the uncertainty in the whole of MARC was to have been performed, for a release of a few nuclides, as an input to the MARIA code design.

III. Progress achieved:

Work on this project this year has been on the planning of techniques for performing uncertainty analyses with MARC and other ACA codes. Consideration has also been given to the availability of data to express the uncertainty on certain input parameters.

Uncertainty analyses on certain modules of the MARC code have already been performed, notably on the Food and Atmospheric Dispersion Modules, and these have been described previously. To enable the uncertainty on the Food Module to be analysed, a detailed and lengthy program was written to enable the food concentration data libraries to be generated within each run of MASRC; in other words, within each run of the MARC programs, sampling was performed on the ranges of the basic input data to the foodchain models.

Because of the complexity of the whole MARC code and its supporting programs, the techniques used in the FOODMARC uncertainty assessment are not appropriate for an uncertainty analysis on the whole code. This would require the construction of a large number of complex programs to enable MARC's input data libraries to be generated within each call of the MARC programs in the analysis. The computing time required would also be greater than is realistic.

For this reason, it has been decided to generate instead input data libraries which contain uncertainty ranges and distributions, and to sample from these libraries in each call of the MARC programs. This requires separate uncertainty studies to be undertaken to ascertain the uncertainty on the data in the libraries. Consideration will also need to be given to the correlations between data in the same libraries, and also to correlations between data in different libraries.

One data library where consideration has been given to the quantification of the uncertainties is that for doses per unit intake of radionuclides. This uncertainty arises from the uncertainty on the basic parameters used in metabolic and dosimetric models. As a first step to obtaining ranges and distributions on the uncertainty of this data, probability distributions for the variability of doses per unit intake in a population have been determined for several radionuclides of significance in accidental releases. This work will be extended to estimate the uncertainty on the best-estimate of the dose per unit intakes for a range of radionuclides.

IV. Objectives for the next reporting period:

The work in the next reporting period will centre on the need to finalise the models for the MARIA package. The uncertainty analysis of the entire MARC code will be finalised, including the derivation of uncertainty data for the data libraries of MARC. The derivation of these data libraries for uncertainty analyses will also be directly applicable to the COSYMA code.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Kernforschungszentrum Karlsruhe GmbH
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Postfach 3640
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Bundesrepublik Deutschland

VI. Publications:

1. Jones, J A and Hill, M D. Uncertainty analysis of the atmospheric disperion module of MARC. IN proceedings of the joint CEC/OECD(NEA) workshop on recent advances in reactor accident consequence assessment, Rome, January 1988. Report EUR 11408 (1988).

2. Jones, J A, Brown J and Hill, M D. Uncertainty analysis of reactor accident consequence assessments. In Transactions of the American Nuclear Society, Vol. 56, p 358 (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen- und Umweltforschung mbH
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BI6-F-111-D

Head(s) of research team(s) [name(s) and address(es)]:

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Institut für Strahlenschutz
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Ingolstädter Landstrasse 1
D-8042 Neuherberg

Telephone number: (89) 31.872.216

Title of the research contract:

Quantification of radiation risks, optimization procedures and analysis of occupational exposure.

List of projects:

1. Somatic radiation risks and optimization procedures.
2. Assessment of external and internal exposures.
3. Assessment of occupational exposure.

Title of the project no.: 1

SOMATIC RADIATION RISKS AND OPTIMIZATION PROCEDURES

Head(s) of project: W. Jacobi and H.G. Paretzke

Scientific staff: D. Chmelevsky, M. Gerken, K. Henrichs
H.G. Paretzke, W. Jacobi

I. Objectives of the project:

a) Risk assessment for radiation carcinogenesis at low doses from epidemiological data and other pertinent information:
- improvement of the quantification of exposure-time-effect surfaces for radiation induced late effects in man at low doses and dose rates,
- testing of the quantitative statistical methods for their operation characteristics in the presence of confounding variables,
- development of mechanistic models for radiation carcinogenesis for selected tumor types.

b) Evaluation of procedures for the optimization of radiation protection.

II. Objectives for the reporting period:

a) - Derivation of risk estimates for the remaining tissues,
- Quantification of the influence of the revision of the A-bomb dosimetry on the estimated risks.
- Continuation of the evaluation of the CEA-rat-experiments for other cancer types.
- Clarification of the influence of the length of the application period of Ra-224 on bone sarcoma incidences.

b) - Evaluation of optimization strategies applied in decisions of private life,
- analysis of risk reduction strategies employed in limitation of risks from chemical pollutants.

III. Progress achieved:

Methodology:

a) - Numerous epidemiological studies on populations exposed to ionizing radiation (A-bomb survivors, patients, occupationally exposed workers) were reviewed taking into account possible biases, confounders, and statistical and dosimetric uncertainties. For the derivation of dose-rate reduction factors several models for carcinogenesis were analysed.

- Within the framework of the analysis of animal experiments, a collaboration was started with Prof. Broerse (TNO) to insure that similar methods will be used to analyse comparable data; computer programs have been exchanged and tested.

- Radon daughters inhalation and neutron or gamma whole body irradiation were compared in their efficiency at low doses to produce lung carcinomas in Sprague-Dawley rats.

- The epidemiological study on the German patients treated with Ra-224 was continued in collaboration with the Kinderpoliklinik der Universität München.

- Various mathematical models that predict exposure-time-risk surfaces based on biological assumptions for carcinogen-induced tumors were critically reviewed.

b) Because of changed priorities (due to Post-Chernobyl activities) in this year optimized radiation protection strategies for the usage of highly contaminted agricultural products were evaluated.

Results:

a) - Estimates of radiation induced risks are now available for bone marrow, breast, GI-tract, lung, skeleton, skin, thyroid and for the "remaining tissues". The epidemiological observations do not allow a final decision concerning the dose-effect relationship. So, our results presented in Table 1 are given for both the linear and the linear-quadratic model. For the temporal extrapolation of observed risks to life-time risks the absolute risk model was adopted for leukemias and bone sarcomas, while for all other cancers the relative risk model was used.

Table 1: Somatic lifetime risks per 10^4 per Gy for assumed linear (L) resp. linear-quadratic (LQ) dose response relationships.

Organ	Model	LQ	L
Bone marrow	A	21	52
Bone	A	1	1
Breast*	R	80	80
Lungs	R	36	90
GI-Tract+	R	90	224
Thyroid	A/R	17	17
Others	R	15	38
Total		260	502

* including men
\+ Stomach, Colon, Liver, Pancreas
A=absolute time projection model
R=relative time projection model

- Multi-stage models provide a useful framework for describing quantitative aspects, such as time- and age-dependent exposure, the determination of early-, intermediate- and late-stage carcinogens, and the consequences of additive and multiplicative effects, but without specifying the nature of the underlying cellular processes and structures and without considering modifying factors and growth periods. Multi-stage models with limited numbers (2 or 3) of stages include a variety of parameters (cell-loss, proliferative advantage, time distribution of tumor-growth etc.) and generally apply to a special tumor-type, but do in detail and special cases disagree with epidemiological or experimental data.
- According to Preston and Pierce the revision of the dosimetry among the A-bomb survivors results in risk estimates which are - according to them - about 40% higher than earlier; these risks are estimated on the basis of the colon doses as measure of exposure.
On the other hand, RERF, Hiroshima, reports that the risk estimates appear to remain unchanged by the revision (RERF rep. 11); however the evaluation of the data is not yet complete.
- The analysis of the data from the epidemiological study of the ^{224}Ra-patients is being continued. It has been claimed years ago by C. W. Mays, NCI, that ^{224}Ra had a greater effectiveness in inducing bone sarcomas when given over a longer period of time. This important point which had been analysed on the basis of less complete data than the present data and with methods which did not sufficiently correct for several confounding factors has been reanalysed.

It appears that the earlier conclusions of Mays were essentially correct. Rank-order tests devised to correct for the correlation between dose and injection span and dose and age at injection indicate a positive effect of the injection period which was significant on the 5% level. The quantitative analysis showed consistence with the following assumed relation between cumulative rate, dose and injection period:

$$R(t,D,\tau) = R_s(t)*(D + 0.23*D^2) * \exp(-0.52*\frac{D}{\tau})$$

where the mean skeletal dose D is given in Gy and τ is the injection duration in months. The base-line function $R_s(t)$ is well approximated by a log-normal function of the time since beginning of the injection.

b) After large environmental contaminations, the introduction of various activity limits for agricultural products used for human nutrition is being considered in countries of the EC. We analysed the applicability and efficacies of various strategies for the usage of agricultural products with contaminations above these limits as animal feed in such a way that the final contamination of the respective animal end-product (e.g. milk, meat, eggs) are below those limits for human nutrition. Here the nuclides Sr-90, I-131, Pu-239, Am-241, and Cs-137 were considered, and various feeding procedures could be identified were agricultural products with contaminations more than ten times the limits for human consumptions could safely be used as feed. The results of this work formed also the base of a respective recommendation of the German Strahlenschutzkommission (published in Bundesanzeiger No. 208, 5.11.88, p. 4758-60).

Discussion:

a) - The GI-tract could contribute about 50% to the total risk. This is due to the method adopted for the transport of risk estimates between different populations, namely by assuming that the absolute number of radiation induced cancers does not vary between different populations. In contrast to the relative contributions of each tissue, the total risk is nearly independent of the method chosen. This result is in agreement with those of the BEIR-III study and the approach used in the American "Radioepidemiological Tables" of the National Institutes of Health.

   - The increase reported by Preston and Pierce might be mainly due to their use of the colon dose as measure of exposure. As the evaluation by RERF is based on organ specific doses it may be concluded, that the risk estimates for the A-bomb survivors will remain unchanged by the revision of the dosimetry; the evaluation of the remaining group of exposed people is not expected to result in significant changes. However, because of the lack of knowledge of the original data more work needs to be done to confirm this conclusion.
   - The category of tolerance-distribution models has gained wide acceptance in chemical carcinogesis, but contradicts the postulate of the carcionogenic process being stochastic.
   Models that regard the role of the immune-system, repair processes, tumor-regression or heterogeneity of populations have not yet been developped.

b) It remains doubtful whether single- or multi-criteria decision making in the optimization of radiation protection measures will lead to a higher acceptability of the minimized total detriment strategy. However, the assumptions and weight-setting underlying a decision process will become more transparent. It is important to assess also the actual feasability of an optimum strategy. In the context of contaminated agricultural products, it must be expected that in a given situation the public market and political considerations might lead to different settings of weights and thus to the realization of different decisions.

IV. Objectives for the next reporting period:

a) - final analysis of the experiments performed by Lafuma et al. (in cooperation with J. Broerse et al.).
   - analysis of the incidences of various neoplasms in Sprague-Dawley rats at low γ- and n-doses; derivation of RBE-values.
   - further analysis of new data from RERF, Hiroshima, to clarify the actual effect of the new dosimetry on derived risk factors.
   - evaluation of radiation carcinogenesis models regarding the dose rate reduction factors for various biological end points.

b) Final Report on possibilities and limitations of quantitative methods for the optimization of radiation protection and on risks of the daily life in various European countries as a yardstick.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Prof. Gössner (GSF) and other Members of EULEP, C.E. Land (National Cancer Institute - Bethesda), National Radiological Protection Board, Harwell (Drs. Ennis, Kendall et al.), CEN-FAR, Fontenay-aux-Roses (Drs. Lafuma, Parmentier, et al.), Prof. A.M. Kellerer (Univ. Würzburg), Prof. H. Spiess (Univ. München).

VI. Publications:

D. Chmelevsky, C.W. Mays, H. Spiess, F.H. Stefanie, A.M. Kellerer:
An epidemiological assessment of lens opacifications with impaired vision in patients injected with Radium-224.
Radiat. Res. 115,238-257,1988.

D. Chmelevsky, A.M. Kellerer, C.E. Land, C.W. Mays, H. Spiess:
Time and dose dependency of bone-sarcomas in patients injected with Ra-224.
Radiat. Env. Biophys. 27, 103-114, 1988.

J. Lafuma, D. Chmelevsky, J. Chameaud, R. Masse, M. Morin, A.M. Kellerer:
Pulmonary Carcinomas in Sprague-Dawley rats after exposure to low doses of radon-daughters, fission-neutrons or gamma-rays.
Radiat. Res., to appear in May 1989.

D. Chemelevsky, C. W. Mays, H. Spiess, F. H. Stefani, A. M. Kellerer:
The cataract response in Radium-224 patients.
Brit. J. Radiol., to appear 1989

H.G. Paretzke:
Risiko für somatische Spätschäden durch ionisierende Strahlung.
Phys. Bl. 45, 16-24, 1989.

Title of the project no.: 2

a) Assessment of external exposures of members of the public after accidental releases of gamma emitters from industrial facilities
b) Assessment of internal exposures due to incorporated radionuclides for members of the public.

Head(s) of project:

W. Jacobi and H.G. Paretzke

Scientific staff:

P. Jacob, K. Henrichs, H. G. Paretzke,
R. Meckbach, W. Jacobi

I. Objectives of the project:

a)- Calculation of organ doses in certain homes and in open air for various relevant gamma emitters in the soil and sitting on walls of constructions by means of Monte-Carlo methods,
- Check of the accuracy and reliability of simplified calculation methods,
- Comparison of computed results with experimental data.
b)- Calculation of organ-specific exposure rates for the internal exposure due to consumption of contaminated foodstuffs (elements: Sr, Tc, Cs, I, U, Pu, Am, Np, Cm) by members of the public,
- Assessment of the reliability and variability of these dose-factors.

II. Objectives for the reporting period:

a) Evaluation of gamma-spectra which have been recorded in houses during the first days after the deposition of Chernobyl radionuclides in Munich and during a shielding experiment at Cadarache (France);
comparison of measured data with photon fluences calculated by Monte-Carlo simulations.

b) - revision of dose calculations according to new dosimetric models describing bone structures presently developped by ORNL;
- search for new biokinetic data especially for the application of dose conversion factors for members of the public.
- identification of nuclides for which data are urgently required.

1. Methodology

a) The kerma in selected homes and in open air for gamma emitters with different source energies deposited on the environment and on the house has been determined by detailed Monte-Carlo photon transport calculations. For unfolding measured spectra the Monte Carlo method has also been used to simulate the photon transport in a high purity Germanium detector, with which the gamma spectra inside houses have been recorded under different radiation conditions.

b) Publications were reviewed to derive biokinetic data for relevant radionuclides.
For the interpretation of whole body measurements performed after the Chernobyl accident the biokinetics of dietary Cs were followed in a group of volunteers (measurements of administered activity, urinary excretion, whole body activities) and the measured data were approximated by optimization of parameters of a biokinetic model.

2. Results

a) The results for external exposures due to radionuclides deposited in urban environments were expressed in the form of so-called location factors, which characterize the exposure at a given location relative to the exposure over a lawn for the same deposition event. Fig. 1 shows for the case of a terrace-house, that location factors for well shielded locations depend strongly on photon energy. The basement is for a source energy of 300 keV 10 times better shielded than for a source energy of 3 MeV, whereas the difference of the exposures in the living rooms in only a factor 2.
A method has been developed to unfold the measured photon spectrum of a high purity Germanium detector. It allows to determine the real photon spectrum at the measuring site from the measured spectrum, which includes all effects of the interactions in the Germanium cristal, i.e. not only the photo effect. This is necessary to assess the contribution of scattered photons to organ doses in shielded locations also from in-situ measurements.

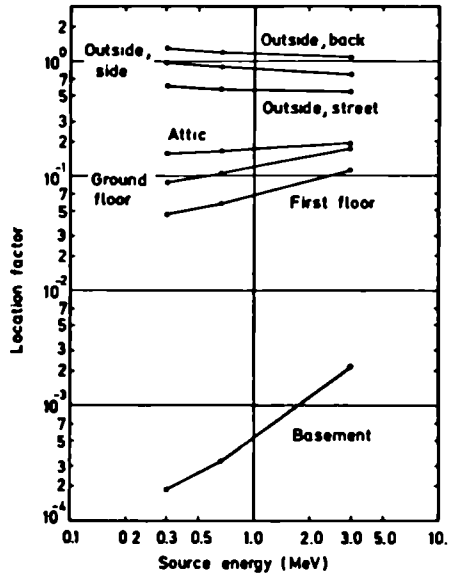


Fig. 1: Location factors as a function of source energy for a row of terrace-houses in an urban environment after a dry deposition. The lines drawn in the figure serve only to guide the eye.

b) - In contrast to our expectation ORNL did not yet publish revised absorbed fractions for the skeleton.

- The measured biokinetic parameters of dietary Cs may be well described by a two-compartment model; the derived half-lives of the long term component vary between 50% and 200% of the corresponding ICRP value. The observed short term retention is much faster than assumed by ICRP; this may be explained by a faster resorption from the small intestine into the transfer compartment. The observations do not show correlations between total body potassium contents and Cs metabolism.

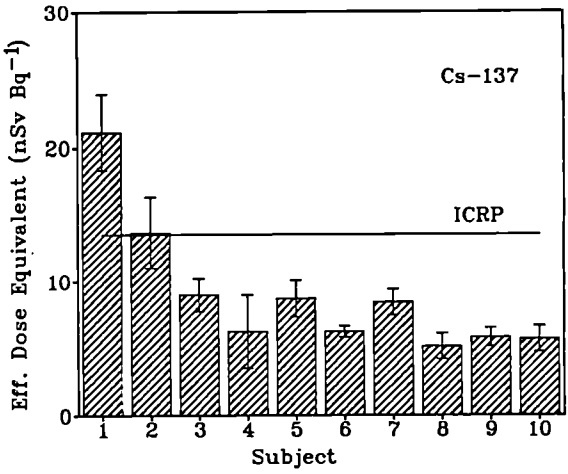


Fig. 2: Dose conversion factors for ^{137}Cs derived from measured bio-kinetic data for dietary Cs.

3. Discussion

a) The measured data on shielding of houses with respect to gamma irradiation published in the literature have the shortcoming, that either simple source geometries are used which do not describe the situation of the contamination of the environment after an accidental release of radionuclides or that only dose rates have been measured, which do not allow a direct determination of the shielding. The method developed in the past year (see sect. 2a) together with measurements of gamma spectra inside and outside houses will resolve this shortcoming and therefore it will be possible to test the calculated location factors in a situation of a contamination of the whole environment of different houses.

b) - our results fit well into the data base available showing that the model adopted by the ICRP is adequate for dosimetric purposes but not for the description of the individual Cs retention.
   - The review of published biokinetic data shows that for some important radionuclides metabolic data are urgently required and further experimental research is needed especially for the derivation of f_1-values as functions of age, chemical form and dietary habits.

IV. Objectives for the next reporting period:

a) The results obtained will be summarized in a model for the assessment of external exposures due to radionuclide depositions in urban environments.

b) In a final report the calculated dose conversion factors for...., and, as far as possible, their variability will be summarized as functions of age and time since incorporation. Radionuclides for which further research is needed will be identified.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

NRPB-Chilton (Drs. Dennis, N. Adams), CEA-Fontenay-aux-Roses (Dr. Parmentier), Riso-Laboratory (Dr. Hedemann Jensen), Universität Toulose (Prof. Blanc), ORNL-Oak Ridge (Dr. Eckermann).

VI. Publications:

Jacob, P., Paretzke, H.G., Rosenbaum, H., Zankl, M.: Organ doses from radionuclides on the ground. Part I: Simple time dependences. Health Physics 54, 617-633 (1988)
Jacob, P., Paretzke, H.G.: Organ doses from radionuclides on the ground. Part II: Non-trivial time dependences. Health Physics, 55, 37-49 (1988)
Meckbach, R., Jacob, P., Paretzke, H.G.: Abschirmung von Gammastrahlung durch Gebäude. Proc. IVth European Congress/XIIIth Regional Congress of International Radiation Protection Association (IRPA), Salzburg, Österreich, pp. 153-157 (1988)
Meckbach, R., Jacob, P., Paretzke, H.G.: Gamma Exposures due to Radionuclides Deposited in Urban Environments. Part I: Kerma Rates from Contaminated Urban Surfaces. Accepted for publication by Rad. Prot. Dos.
Meckbach, R., Jacob, P.: Gamma Exposures due to Radionuclides Deposited in Urban Environments. Part II: Location Factors for Different Deposition Patterns. Accepted for publication by Rad. Prot. Dos.

Title of the project no.: BI6-F-111-D

QUANTIFICATION OF RADIATION RISKS, OPTIMIZATION PROCEDURES AND ANALYSIS OF OCCUPATIONAL EXPOSURE

Head(s) of project:

Dr. G. Drexler

Scientific staff:

Dr. D.F. Regulla
Dipl.-Phys. J. David
Dipl.-Phys. H. Eckerl
Dipl.-Ing.(FH) H.-N. Brand
Dr. G. Drexler

I. Objectives of the project:

- Development of personal and partial body dosemeters; performance of laboratory and field tests.
- Workplace analysis, with particular reference to the recent ICRP statement at Paris, France in July 1985 (NRPB Radiol. Prot. Bull. 65, 1985) and interpretation of measured doses in terms of risk relevant quantities.
- Assessment of personal doses for individuals and collectives. Statistical analysis of occupational exposures and evaluation of trends.
- Development of strategies for optimization of radiation protection.

II. Objectives for the reporting period:

- Performance of field tests of a prototype partial body dosemeter according to present standard requirements.
- Continuation of development of a combined film/TLD badge and of a work place specific beta dosemeter system for reactor personnel.
- Continuation of compilation and statistical analysis of personal dosimetry data from the GSF Personnel Monitoring Service. Assessment of individual lifetime doses and exposure histories.
- Continuation of the evaluation of exposure conditions and dose distributions in angiography, nuclear medicine, and tentatively nuclear industry.

III. Progress achieved:

Workplace Analysis: In diagnostic radiology emphasis was given to angiographic techniques, particularly digital subtraction angiography. The frequency of this type of examination is increasing. Further, due to extended time of examination and presence of almost all staff close to the patient, the radiation exposure for the involved persons can become remarkable. To evaluate workplace-specific exposure models measurements were made with selected staff of physicians and assistants at (a) the Herzzentrum München and (b) the Krankenhaus Kronach. They agreed to wear, additional to the usual personal dosemeter, seven types of partial body dosemeters, which were partly designed for this study: The following dosemeters belong to one set: (a) two TLD rings for the right and the left hand, (b) one plastic button, containing a TLD-chip, which was fixed by adhesive tape near the eye-lenses, (c) two small plastic boxes, both filled with four of the above described plastic buttons, one of them to be worn beside the usual personal dosemeter, the other outside the apron at the collar, (d) furthermore, two wristlet dosemeters, transformed from TLD rings for the right and left hand.

First results show that the personal dosemeter covered by the apron indicates nearly always an exposure below 0.1 mSv. The dose to the collar or the head, however, can be remarkably higher for physicians and assistants working close to the patient. The measurements revealed monthly doses at the collar between 0.3 mSv and 3.0 mSv and two extreme values of 9.5 mSv and 11 mSv.

The measurements showed that for the physicians in almost all cases the exposure to the left hand was significantly higher than to the right hand. The monthly values for the right hand ranged from 0.3 mSv to 9.1 mSv whereas for the left hand doses between 1.0 mSv and 14.0 mSv were measured. Again two extreme values of 67 mSv and 90 mSv were found (together with the high doses measured at the collar and the head).

No significant correlation between the doses measured at the hand and the wrist could be found; ratios of "ring dose" versus "wrist-dose" ranged from 1 to 10. Hence, the measurement at the wrist can usually not deliver a reliable estimate for the extremity dose.

Work place specific beta dosemeters for reactor personnel: The objective of this study was to (a) identify relevant beta fields and (b) to evaluate qualitative and quantitative figures on source and field parameters. The figures have been considered to be the basis for requirements for a personal dosemeter capable to detect beta radiation and allow for correct dose assessment also in mixed photon-beta fields. Measurements were made at a BWR during both the operational and maintenance phase using survey meters with ion chamber technique, GM counter, film and TL dosimetry. Preliminary results revealed dose rates up to around 0.2 Sv/h, resulting from extended contaminations and beta energies of around 0.5 MeV. Similar experiments were started in fuel element production applying different integrating dosemeter types for comparison. Results will be reported at a later stage.

Partial body dosemetry system: As for the equipment most components were developed and transferred to industry for prototype production. As part of the internal quality control on the system uncertainties, thirty dosemeters were exposed to unknown doses from 1 mSv to 500 mSv Co60 gamma radiation; as a result coefficients of variation were found within about 5 %. The lower detection threshold was around 0.07 mSv; doses of 0.1 mSv could be determined with a coefficient of variation of better than 30 %. The system was involved into the annual PTB quality control usual in personal monitoring with unknown dose equivalent between 0.1 mSv and 1 Sv in the energy range from 10 keV to Co60 gamma radiation. Apart from the mean value of 1.30, a remarkable coefficient of variation of 18.5 % was achieved.

The new partial body dosemetry system was also subject of a half-year field test with a monthly evaluation; fourty institutions were involved with more than 240 persons from different areas, i.e. medicine, industry and nuclear field. The results of the first control period revealed a collective dose of 128 mSv or a mean dose per person of 0.72 mSv. 85 % of the doses were below 1 mSv, 0.6 % above 10 mSv.

IV. Objectives for the next reporting period:

- Continuation of a multi-user field test of a prototype partial body dosemetry system to optimize construction, handling, performance and operation of the readout equipment.

- Continuation of development of a) a combined film/TLD badge and b) a work place specific beta dosemeter system for reactor personnel.

- Continuation of compilation and statistical analysis of dosimetry data from the GSF Personnel Monitoring Service.

- Continuation of work place analysis in radiological industry.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

David, J.: Monitoring of beta radiation in operational dosimetry in industrial enterprises. In: Radiation Protection 38, Beta Dosimetry, CEC, Luxembourg, 192-195 (1988)

Drexler, G., Eckerl, H.: On the influence of the exposure model on organ doses. Transactions of the Am. Nucl. Society and the Europ. Nucl. Society, TANSAO 57 1-584, 215-216 (1988)

Drexler, G., Eckerl, H., Haid, G., Scheibe, D.: Statistische Ergebnisse aus der amtlichen Personendosisüberwachung 1987 - Auswertungsstelle für Strahlendosimeter der GSF. GSF-Bericht 21/88 (1988)

Regulla, D.: Beta radiation fields relevant to radiation protection. In: Radiation Protection 38, Beta Dosimetry, CEC, Luxembourg, 165-176 (1988)

Zankl, M., Veit, R., Williams, G.*, Schneider, K.**, Fendel, H.**, Petoussi, N., Drexler, G.: The construction of computer tomographic phantoms and their application in radiology and radiation protection. Radiat. Environ. Biophys. 27, 153-164 (1988)
*Medical College of Ohio, Toledo, USA
**Dr. von Haunersches Kinderspital, Univ. München

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-113-DK

Technical University of Denmark
Laboratory of Applied Physics I
DK-2800 Lyngby

Head(s) of research team(s) [name(s) and address(es)]:

Dr. N. Jonassen
Laboratory of Applied Physics I
Technical University of Denmark
DK-2800 Lyngby

Telephone number: (2) 88.24.88

Title of the research contract:

Investigation and development of methods to control the level of radon daughters in indoor air.

List of projects:

1. Investigation and development of methods to control the level of radon daughters in indoor air.

Title of the project no.:

BI6-113-DK
INVESTIGATION AND DEVELOPMENT OF METHODS TO CONTROL THE LEVEL OF RADON DAUGHTERS IN INDOOR AIR.

Head(s) of project:

Dr. Niels Jonassen

Scientific staff:

Bent Jensen
Younes Leroul

I. Objectives of the project:

It is the objective of the project to study especially those characteristics of airborne radon daughters which have a direct or indirect influence on the radiological effectiveness of remedial aircleaning techniques, such as filtration and electrostatic deposition.

II. Objectives for the reporting period:

During the period covered in this report the main emphasis of the work has been put on the following:

A) Plateout studies.
The plateout of individual radon daughters on metal discs exposed to "deformed" negative electric fields has been studied together with the effect of the plateout on the remaining airborne, attached and unattached radon daughters.

B) Electrostatic plateout and/or filtration.
The effect of a series of ionizers (commercially available and homemade) on the level and state of the airborne radon daughters has been studied especially in combination with various types of electrofilters.

III. Progress achieved.

A) Plateout studies.

The plateout of individual radon daughters on metal discs exposed to "deformed" negative electric fields has been studied together with the effect of the plateout on the remaining airborne, attached and unattached radon daughters.

The results are very similar to those reported earlier (1987 Progress Report) for positive ionization, except for the fact that the effect of positive ionization on exposure and dose is about two times the effect of negative ionization.

B) Electrostatic plateout and/or filtration.

The experiments over the effect of ionizers and electrofilters on the level of airborne radon daughters were carried out in the same (150 m^3) room as described in the earlier progress reports.

With all experiments the following quantities were measured: radon and individual daugther concentrations, unattached fractions of the individual daughters and (normally) aerosol concentration and mean size.

On basis of these results the exposure rate (potential alpha energy concentration) and the mean bronchial epithelial dose rate are calculated.

1. Methodology

The dose(rate) is calculated from the attached and unattached potential alpha energy concentrations rather than from the individual daughter concentrations as was the case for the results reported earlier.

The reason for this change in method is the recent development in dose calculation theories as developed in A. C. James "Lung Dosimetry", in Nazaroff and Nero (Eds.): "Radon and Its Decay Products in Indoor Air", Wiley, 1988.

The conversion factors used are $169 \cdot 10^{-5}$ (Gy/year)/(Bq/m^3 EER) for unattached daughters and $10.15 \cdot 10^{-5}$ (Gy/year)/(Bq/m^3 EER) for attached daughters corresponding to an aerosol AMD of 0.1 um (and assuming a growth in diameter of 50 % in the nose and the upper bronchi).

For each filter and/or ionizer is calculated the percentage of the dose and exposure in the untreated air remaining when the device is operated.

2. Results

The preliminary measurements were performed at low aerosol concentrations.

The results are summarized in Figure 1, where the codes for the filters and ionizers are the following.

Filters:

A: Elixair 1100 (Finnish), B: Elfi 2000 (Swedish), C: Elfi 62, D: Elixair 400

Ionizers:

A: LAP +18 kV (homemade), B: Scanmatic +10 kV.

Ionization/Filtration unit: NORAD (US)

The ionization/filtration unit NORAD is a (professional) device, which can be operated as an ionizer or (mechanical) filter or both simultaneously.

The four emitters, which in one case is operated in combination with the filter B, are the metal points mounted 1 m below the ceiling as described in 1987 Progress Report.

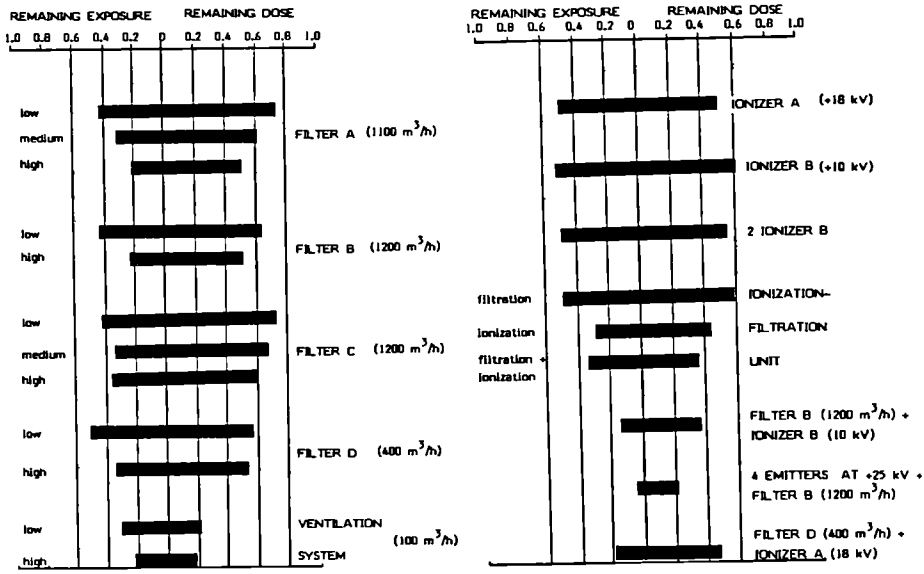


Figure 1. Effect of ventilation and filtration on radon daughter exposure and dose.

The figure shows that the filters at high flow rate give a remaining exposure between 23 and 36 % and a remaining dose between 51 and 58 %. The ionizers alone reduce the exposure to about 50 % and the dose to about 50-60 %.
The NORAD system will, when both ionization and filtration is applied, reduce the exposure to about 30 % and the dose to about 40 %.
It is also evident that the combination of filters and ionizers gives the lowest remaining exposure and dose, the Filter B in combination with 4 emitters at +25 kV thus reducing the exposure to 5 % and the dose to 20 %.

The trend from the preliminary results were pursued in a series of measurements at medium to high aerosol concentrations. These investigations are not yet finished but the results obtained so far are summarised in Table 1. The ionizer used was in all cases the LAP +12 kV model.

Discussion
The results seem to suggest that filtration alone will only give modest reductions in exposure and dose at acceptable filtration rates.
It seems likely that ionization even with rather small ionizers may be as effective as filtration.

UNIT	FILTRATION ALONE		FILTRATION + IONIZATION WITH LAP-ION A	
	REMAINING		REMAINING	
	EXPOSURE	DOSE	EXPOSURE	DOSE
ELFI 62, LOW	0.57	0.87	0.14	0.34
ELFI 62, MED	0.55	0.59	0.16	0.48
ELFI 62, HIGH	0.37	0.44	0.09	0.32
ELIXAIR400, LOW	0.65	0.81	0.15	0.36
ELIXAIR400,HIGH	0.63	0.77	0.22	0.40
ELIXAIR700, MED	0.44	0.67	0.19	0.28
ELIXAIR700,HIGH	0.41	0.50	0.14	0.37
LAP-ION A			0.27 - 0.35	0.33 - 0.46

Table 1. Effect of filtration and ionization/filtration on radon daughter exposure and dose.

It also appears that a combination of ionization and filtration is the most effective use of the devices investigated, leading to a remaining exposure and dose of about 20 (or less) and 30-40 %.

An important question, which partly falls outside the scope of this experimental investigation, is whether exposure or dose gives the best measure of the radiological properties of the air.
All dose calculations are based on assumptions which are poorly verified experimentally, while on the other hand the exposure is an experimentally determined quantity.
It would be short-sighted to base an evaluation of any remedial technique solely on the effect on the calculated doses.

IV. Objectives for the next reporting period:

In the next reporting period the actual plateout of radon daughters, with and without ionization, will be studied with the use of nuclear track films, which will be processed and counted at University College, Dublin.
The effect of more than one ionizer working simultaneously and being placed in various places will be examined, and we will further study any possible unwanted effects of the ionizers such as electrostatic chargings of persons and insulated objects.
And finally it will be attempted to develop a practical combination of a filter and ionizer unit.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

dr. J. P. McLaughlin, University College, Dublin, Ireland.

VI. Publications:

1. Jonassen, N., "Ions, Electric Fields and Radon Daughters, Effect of Filtration and Electric Plateout", Radiation Protection Practice, vol. I, p. 377-380, Pergamon Press, 1988.
2. Jonassen, N. and Bent Jensen, "Removal of Radon Daughters by Filtration and Electrostatic Plateout", Proceedings EPA, The 1988 Symposium on Radon and Radon Technology, Denver, 17-21 Oktober 1988, Session VII, paper 11.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Kernforschungszentrum Karlsruhe
GmbH, KfK
Postfach 3640
D-7500 Karlsruhe 1

Contract no.: BI6-F-128-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. G. Kessler
Inst.f. Neutr.phys.u. Reaktortechn.
KfK
Postfach 3640
D-7500 Karlsruhe 1

Telephone number: (7247) 82.24.40

Title of the research contract:

Methodology for evaluating the radiological consequences of radioactive effluents released in accidents.

List of projects:

1. Atmospheric dispersion and deposition in off-site accident consequence modelling.
2. The assessment of exposure due to deposited material.
3. Countermeasures to reduce the impact of accidental releases of radioactive materials.
4. Uncertainty analysis.

Title of the project no.: 1

Atmospheric dispersion and deposition in off-site consequence modelling

Head(s) of project:

J. Ehrhardt

Scientific staff:

H.-J. Panitz

I. Objectives of the project:

The overall objective of the project is to develop improved and more comprehensive models for predicting rates and patterns of dispersion and deposition following accidental releases of radionuclides to the atmosphere. These models have to be capable of being included in probabilistic accident consequence assessment (ACA) codes. The work forms part of the MARIA2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

Completion of the near and far range atmospheric dispersion models implemented in UFOMOD (MUSEMET, ISOLA) or modified for usage as stand-alone modules (RIMPUFF, MESOS) with respect to their distribution as parts of the accident consequence code system of project MARIA (to be called COSYMA).

III. Progress achieved:

After extensive comparative deterministic and probabilistic benchmark calculations with different types of atmospheric dispersion models performed at KfK during the last years, a new concept of atmospheric dispersion modelling has been established in the new program system UFOMOD . It fundamentally differs from other accident consequence codes in making use of trajectory models instead of straight-line Gaussian models. Different ranges of validity are distinguished and assigned to the respective trajectory models MUSEMET or RIMPUFF (near range $\lesssim$ 50 km) and MESOS (far range $\gtrsim$ 50 km).

During the reporting period, the above-mentioned computer programs have been completed, tested and applied in various accident consequence assessments for PWRs and a reprocessing plant. Comparative investigations with RIMPUFF and MESOS in the distance range between about 100 km and 250 km based on dispersion calculations along windfields derived from the sets of synoptic data belonging to each model showed a remarkable good agreement of results.

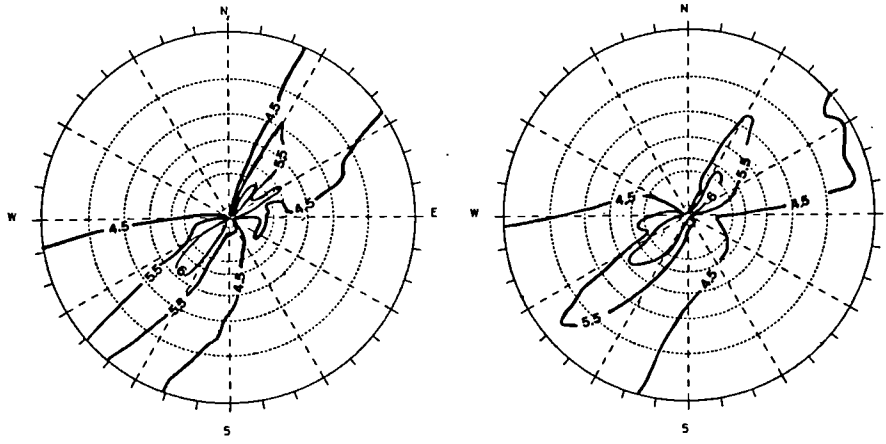
The segmented-plume model MUSEMET has been improved by the implementation of up-dated models for thermal plume rise and the influence of buildings on dispersion of released material including lift-off criterion. The method of calculating cloud gamma doses has been refined by the use of correction factors for the near range derived from Monte-Carlo-calculations for finite and semi-infinite clouds performed at GSF, Neuherberg.

The preprocessing program for meteorological sampling of weather sequences to be considered in generic risk studies has been made more flexible to allow an easy change of the categorization scheme.

An one week workshop on "UFOMOD and the development of an European accident consequence code" has been organized at KfK. To provide an efficient introduction into UFOMOD and to start an early dialogue with potential users of COSYMA, user's guides, model descriptions and illustrative input/output examples of the atmospheric dispersion models were compiled in several documents and distributed during the workshop as supplement to the oral presentations

Recent studies on the source terms showed that accidental releases might occur which extend over time periods from about four days up to several months. Although Gaussian-like trajectory models are potentially able to simulate the atmospheric dispersion during a long-term release the computer time required would be very large. On the other hand, if the release duration is sufficiently long, different atmospheric dispersion conditions during the release can be evaluated statistically by classifying the wind directions, the wind speeds, diffusion categories, and precipitation intensities. In those cases, the fast running straight-line Gaussian model ISOLA developed at KfK can be applied to calculate the time-integrated air concentrations and ground contamination patterns according to the meteorological conditions represented by each of the statistical classes. Afterwards all concentrations are summed up taking into account the frequency of each class. A modified version of ISOLA has been implemented in UFOMOD; thus all source terms between effluents from normal operation and accidental releases can be considered.

To test this concept a comparison between the Gaussian trajectory model MUSEMET and ISOLA was performed. As an example, the figure below shows the ground concentrations after an 144 hours release plotted in polar diagrams. It can be seen that the results of both models are in good agreement. Thus, the statistical evaluation of rather long atmospheric sequences leads to similar results as the explicit consideration of different dispersion situations by a trajectory model.



Comparison of ground concentrations calculated with MUSEMET (left) and ISOLA for a long duration release (144 h); outer radius r = 87,5 km

IV. Objectives for the next reporting period:

The program package COSYMA will be completed by the atmospheric dispersion model ADMARC and the meteorological sampling program extended for applications with trajectory models, both under development at NRPB. Test runs and example calculations will be performed within a quality assurance procedure. The already available atmospheric dispersion model MOTTE for tritium releases will be modified for inclusion in the COSYMA code package. All modules and preprocessing programs for UFOMOD and COSYMA will be documented.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

National Radiological Protection Board (NRPB) Chilton, Didcot, Oxon OX11 ORQ

VI. Publications:

H.-J. Panitz
Improved atmospheric dispersion modelling in the new program system UFOMOD for accident consequence assessments
Proceedings of the joint OECD (NEA)/CEC Workshop on "Recent Advances in Reactor Accident Consequence Assessment"
Rome, Italy, January 25-29, 1988
Commission of the European Communities, Report EUR-11408 EN (1988)
ISBN 92-825 8424-0, p. 92-102

Title of the project no.: 2

The assessment of exposure due to deposited material

Head(s) of project:

J. Ehrhardt

Scientific staff:

C. Steinhauer, I. Hasemann

I. Objectives of the project:

The aim of this project is to develop or improve models for predicting doses to people following deposition of radioactive material after an accidental release to atmosphere. The exposure pathways considered include: external irradiation from material deposited on the ground and on buildings, transfer of radionuclides through terrestrial foodchains, radionuclide transfer in freshwater bodies and in the marine environment and inhalation of resuspended material. The work forms part of MARIA 2.

II. Objectives for the reporting period:

Completion and test of preprocessing programs, modules, data sets and evaluation programs of UFOMOD, which will also be parts of the code system of project MARIA, to be called COSYMA. Preparation of a seminar on accident consequence modelling at KfK.

III. Progress achieved:

In the beginning of this reporting period, an agreement has been reached with NRPB on the overall structure of COSYMA, the code system of project MARIA (see figure below). The program package will be based on the present version of UFOMOD including all preprocessing programs and modules, completed with features of the NRPB program MARC which are not in UFOMOD, and some further improvements considered to be important.

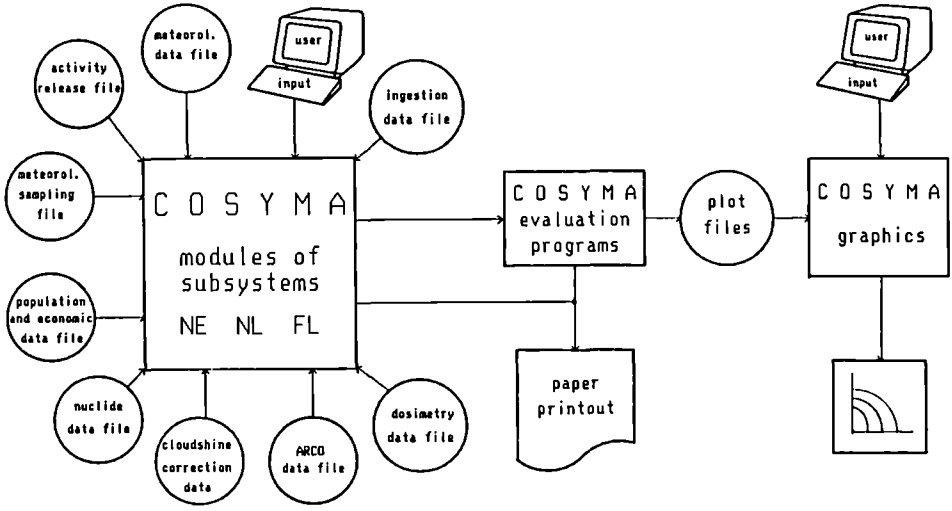
The general features of UFOMOD and technical aspects of its submodels were presented together with example calculations during the "Joint CEC/OECD(NEA) workshop on recent advanced in reactor accident consequence assessment", Rome,Italy, 25 to 29 January 1988, and the "Seminar on UFOMOD and the development of an European accident consequence code", organized by KfK, 17 to 21 October 1988.

The first part of the latter seminar with about 55 participants from 13 countries was intended for potential UFOMOD (and COSYMA) users, and the experience gained from those users who apply the code will be a valuable input for the development of COSYMA. A large number of documents with detailed descriptions of structure, modules, dose and health effects models including countermeasures, input and output, evaluation programs and data sets have been provided during the seminar. In special training sessions a guidance how to use the program for various applications by appropriate choice of parameter values has been given. In the second part of the seminar (one day), all MARIA contractors were asked to present models and data which could be of relevance for COSYMA.

To increase the flexibility of UFOMOD and COSYMA, a set of three special evaluation programs was developed, which allows to correlate nuclide specific activity concentrations with the areas affected, individual organ doses with the number of persons affected, and collective doses with individual doses. The presentation of these results in appropriate diagrams will help to analyse and intepret the results of accident consequence assessments especially at farther distances or for small source terms.

The population grids for Europe partially refined by more detailed data sets at KfK and NRPB have been combined to one updated grid. New population data from Spain have been included The early health effects model has been

modified to allow a more flexible treatment of different dose integration times with corresponding LD_{50}-values and dose thresholds. Lengthy discussion about the modelling of the pulmonary syndrome did not yet lead to a final proposal.



COSYMA: General layout of each subsystem

IV. Objectives for the next reporting period:

The work will be focussed on the preparation of the program package COSYMA for distribution after the CEC workshop in Athens, 1991. This means especially the completion of the methodology by including additional models for the assessment of foodbans via intervention levels based on activity concentrations in foodstuffs, of the health effects from the ingestion pathways with the agricultural production method (collective approximation), and of the effects of radionuclides deposited onto skin and clothes

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

National Radiological Protection Board (NRPB)

Chilton, Didcot

GB - Oxon OX11 ORQ

Gesellschaft fur Strahlen- und Umweltforschung (GSF) mbH

Institut fur Strahlenschutz

Munchen Neuherberg

Ingolstadter Landstr.1

D-8042 Oberschleissheim

VI. Publications:

J EHRHARDT, K BURKART, I. HASEMANN, C. MATZERATH, H.-J. PANITZ, C. STEINHAUER
The new program system UFOMOD to assess the consequences of nuclear accidents
Proceedings of the joint OECD (NEA)/CEC Workshop on "Recent Advances in Reactor Accident Consequence Assessment"
Rome, Italy, January 25-29, 1988
Commission of the European Communities, Report EUR-11408EN (1988)
ISBN 92-825-8424-0, p 27-38

C STEINHAUER, C. MATZERATH, J. EHRHARDT
Method for calculating time dependent individual and collective cancer risks in the accident consequence assessment code UFOMOD
Proceedings of the joint OECD (NEA)/CEC Workshop on "Recent Advances in Reactor Accident Consequence Assessment"
Rome, Italy, January 25-29, 1988
Commission of the European Communities, Report EUR-11408EN (1988)
ISBN 92-825-8424-0, p. 278-288

J EHRHARDT, K BURKART, I. HASEMANN, C MATZERATH, H.-J. PANITZ, C STEINHAUER
The program system UFOMOD for assessing the consequences of nuclear accidents
Karlsruhe, Report KfK-4330 (1988)

C. STEINHAUER
UFOING: Program for assessing the off-site consequences from ingestion of accidentally released radionuclides
Karlsruhe, Report KfK-4475 (1988)

J. EHRHARDT, K. BURKART, I. HASEMANN, C. MATZERATH, H.-J. PANITZ, C. STEINHAUER
Accident consequence assessment modelling in the new program system UFOMOD with illustrative results
Annales de l'Association Belge de Radioprotection, vol. 13, no.3 (1988), p.239-258

Title of the project no.: 3
Countermeasures to reduce the impact of accidental releases of radioactive material

Head(s) of project:

J Ehrhardt

Scientific staff:

D. Faude, I. Hasemann

I. Objectives of the project:

The aim of this project is to quantify the benefit of countermeasures in case of accidental releases to atmosphere in order

- to improve modelling of protective actions in ACA codes and
- to provide a greater input into emergency response planning.

Sheltering, prophylactic evacuation, and evacuation based on measurement of dose distributions in the environment are alternative countermeasures suitable to mitigate or to avoid death due to acute radiation syndrome. In case of alternatives an improved knowledge of risks and benefits tends to facilitate both decision making of the emergency management in a real case and the anticipation of these decisions in risk studies and ACA codes.

II. Objectives for the reporting period:

The main objective of the work in the reporting period was concerned with the completion of UFOMOD, the increase of its flexibility in countermeasures modelling to allow due consideration of strategies and plans existing in various countries, and the development of an economic module for implementation in COSYMA.

III. Progress achieved:

NRPB and KfK are developing a joint model to assess the economic consequences of accidental releases of radioactivity within the framework of the MARIA programme. There have been a number of meetings through 1988 at which collaborative aspects of the model have been discussed. The work described in this progress report is limited to the parts of the work that has been undertaken by KfK.

The aim of the economics model is to calculate in detail the economic consequences of early and late health effects as well as of countermeasures including evacuation, relocation, food bans and decontamination. The following cost categories will be treated:

- Evacuation/Relocation
  - primary costs: expenditures for transport and accomodation,
  - losses of income of people unable to work,
  - losses of the value of land and its assets.

- Food Ban
  - costs of lost produce,
  - costs of lost agricultural land and capital,
  - costs of disposal of food.

- Decontamination
  - costs arising from decontamination procedures.

- Health Effects
  - medical treatment costs,
  - costs arising from an individual's contribution to society.

Through 1988, the interface between the economic model and the relevant other modules of the UFOMOD code system has been investigated, whereas UFOMOD has been taken as a basis for the European code system COSYMA. Especially, the information, that is provided by the other modules as an input for the calculation of economic consequences has been analysed in detail. Preliminary computer test runs have been carried out in order to verify the proper

handling of the data. The monetarization of countermeasure consequences required in some aspects a more precise modelling of the timing and duration of relocation, decontamination and food-bans; the respective parts in the UFOMOD/COSYMA modules are being modelled in more detail.

A second category of input information has to be provided by the user from statistics of the national economy, especially for the calculation of losses of income and losses of land and tangible assets in case of evacuation and relocation Various contacts to Statistical Offices in the Federal Republic in Germany have been established in order to collect this kind of input data, especially on a regional and local basis.

The procedure for calculating the different cost categories mentioned above has been developed in detail, and first considerations have been given to the structure of the economics module to be incorporated in the COSYMA code package.

IV. Objectives for the next reporting period:

Modification of countermeasures modelling to allow further options of early and late countermeasure strategies (e.g. short-term relocation less than 1 year, sheltering without evacuation). Completion, implementation and documentation of the economic module and appertaining data.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

National Radiological Protection Board (NRPB), Chilton, Didcot, Oxon OX11ORQ

VI. Publications:

K. BURKART, J. EHRHARDT, I. HASEMANN
Applications of the new program system UFOMOD in the field of emergency response planning
Proceedings of the joint OECD (NEA)/CEC Workshop on "Recent Advances in Reactor Accident Consequence Assessment"
Rome, Italy, January 25-29, 1988
Commission of the European Communities, Report EUR-11408EN (1988)
ISBN 92-825-8424-0, p.301-311

Title of the project no.: 4

Uncertainty Analyses

Head(s) of project:

Friedmar Fischer

Scientific staff:

I. Objectives of the project:

The aim of this project is, on the basis of applying various techniques available for uncertainty and sensitivity analysis of large computer models, to review and select the techniques which are most appropriate for analyzing the uncertainty in the predictions of accident consequence assessments. The techniques will be used to identify and and characterize major contributors to uncertainty in such assessments. The work forms part of MARIA 2.

II. Objectives for the reporting period:

The main aims of the investigations in 1988 were

- to continue uncertainty/sensitivity analysis on submodel basis for the new program system UFOMOD version NE87/1
  - the atmospheric dispersion and deposition submodel (trajectory model)
  - the countermeasures submodel

  both including special investigations concerning the percentage contribution of sensitive parameters to uncertainty in consequences.
- to study theoretically the 'stability behaviour' of parameter correlation matrices with respect to different parameter distribution functions

III. Progress achieved:

Atmospheric dispersion and deposition submodel (see [3])

Based on a set of about 20 model parameters [1] (i.e. leaving out quantities describing the source term (like thermal energy) or measured values (like wind speed or wind direction), near range concentration fields (on ground surface and in the air near ground) were investigated.

E.g., for caesium concentration on ground and air near ground there was a dominant influence of dry and wet deposition of caesium, VD(AER) and LD(AER), mixing height, (HMIX), and horizontal plume diffusion, SIGZ.

- *Concentration on ground surface*
  - LD(AER) accounts for 54 % to 94 % (from nearer to farther distances)
  - VD(AER) accounts for 39 % to 4 % (from nearer to farther distances)

  of the explained uncertainty in consequences
- *Concentration in the air near ground*
  - SIGZ accounts for 94 % to 87 % (from .875 km to 4.9 km distance), HMIX 9 % (in 4.9 km distance)
  - HMIX accounts about 96 %, VD(AER) only 3 % (in 27 km distance)

  of the explained uncertainty in consequences

Some results were presented in [2].

Countermeasures submodel (see [4])

The area covered by the near range subsystem NE87/1 is chosen in such a manner that exclusively in this area fast protective actions may be necessary and early health effects may occur. As sequential countermeasures in the near range , evacuation of a keyhole shaped area (A) and an angle and/or evacuation of an area (B) determined by an isodose line are modelled.

Based on a set of about 20 model parameters (see Figure 1) the influence of model parameter variations on uncertainty in consequences was investigated with respect to

- acute organ doses (lung, bone marrow) for three distances (.875 km, 4.9 km, 27 km)
- individual risks (lung, bone marrow) for three distances (.875 km, 4.9 km, 27 km)
- number of early fatalities (pulmonary syndrom, hematopoietic syndrome)

Three variables led to a significant influence on variations in consequences: TINA, GRWRTB and PAUFA. Some example results are given:

- *Individual organ doses (bone marrow)*
  - TINA accounts for 86 % to 1 % (from nearer to farther distances)
  - GRWRTB accounts for 15 % to 98 % (from nearer to farther distances)

  of the explained uncertainty in consequences
- *Early fatalities for lung (bone marrow)*

	PARAMETER EXPLANATION
TINA	Initial delay of actions in A
TDELA	Delay between end of release and end of sheltering in A
PAUFA	Fraction of the population with different behaviour during sheltering in A
GRWRTB	Intervention level for evacuation of area B
IEVA2	Index of last outer radius belonging to area A
WGRNZA	Angle of keyhole sector of A
WSHIFT	Azimuthal shift of the sector of area A against wind direction of the 1st release phase
TAUSA	Driving times to leave A

Figure 1. Parameter list of uncertain model parameters for the countermeasures submodel of UFOMOD, Version NE87/1

- TINA accounts for 76 % (17 %)
- PAUFA (outside, rural area) accounts for 5 % (75 %)

of the explained uncertainty in consequences

Some general findings during uncertainty/sensitivity investigations:

- Sensitivity tables not only must contain correlation values (PRCCs) but also the percentage contribution of each parameter to variations in consequences ('coefficients of determination', R^2). For example, very sensitive parameters may have a small R^2 only, because they are dominated by *one* single uncertain parameter.
- For the atmospheric dispersion and deposition submodel two types of a LHS-design were used: one with different parameter distributions, the other with all parameters uniformly distributed.
  Changes in distributions had no effect on the ranks of the most sensitive parameters, but led to changes in minor ranking positions.
- For the countermeasures submodel it was important to show the effect of partly or completely correlated driving time parameters on uncertainty in consequences.

First analyses concerning the 'stability behaviour' of parameter correlation matrices and the effects of distribution changes show:

- consequences may be influenced , if sample values of model parameters are replaced by their respective ranks,
- linearizing of given distributions may led to 'correlation stability', i.e. in these cases raw and rank matrices differ only slightly . This was proofed formally for some examples (see [5]).

IV. Objectives for the next reporting period:

The work on uncertainty analysis will continue on submodel basis using the dose and early health effects submodels in the new program system UFOMOD, version NE87/1 (i.e. aiming at propagation of uncertainties through the submodels). The investigations on the various submodels will then build the basis for an overall uncertainty/sensitivity analysis of the UFOMOD subsystem NE.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

National Radiological Protection Board (NRPB) Chilton, Didcot GB - Oxon OX 11 ORQ

VI. Publications:

[1] J. Päsler-Sauer, "Uncertainty analysis of the atmospheric dispersion model of UFOMOD: Selection of parameter ranges and frequency distributions", Internal Report, Kernforschungszentrum Karlsruhe GmbH, December 1987

[2] F. Fischer, "UFOMOD - Uncertainty and sensitivity analyses", Paper presented at the Joint CEC/OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy, January 25 - 29, 1988

[3] F. Fischer, J. Raicevic, J. Päsler-Sauer, "Uncertainty analysis for the atmospheric dispersion and deposition submodule of the new UFOMOD code system," Kernforschungszentrum Karlsruhe GmbH, to appear as KfK-Report No. 4447, 1989

[4] F. Fischer, J. Ehrhardt, K. Burkart, "Uncertainty analysis for the countermeasures submodule of the new UFOMOD code system," Kernforschungszentrum Karlsruhe GmbH, to appear as KfK-Report No. 4472, 1989

[5] J. Raicevic, F.Fischer, "Some aspects concerning stability of rank and raw correlations obtained by the SANDIA LHS-code", Internal Report (Draft), Kernforschungszentrum Karlsruhe GmbH, September 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-P-114-GR

Greek Atomic Energy Commission
GAEC
NRC "Democritos"
153 10 Aghia Paraskevi
GR- Attiki

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J. Kollas
Nuclear Technology Department
GAEC - NRC "Democritos"
153 10 Aghia Paraskevi
GR- Attiki

Telephone number: 65.10.348

Title of the research contract:

Individual and social radiation risks resulting from the operation of nuclear facilities and assessment of risks derived from enhanced natural and artificial radioactivity in Greece

List of projects:

1. Assessment of the radiation risk in the presence of large population centres from the operation of nuclear facilities for population distribution criteria.
2. Wind flow and dispersion aspects of radiation risk assessment.
3. Investigation of enhanced natural and artificial environmental radioactivity in Greece.

Title of the project no.: 1

Assessment of the radiation risk in the presence of large population centres from the operation of nuclear facilities for population distribution criteria.

Head(s) of project:

Dr. J.G. Kollas

Scientific staff:

Dr. I. Papazoglou, Mr. M. Christou, Ms. E. Daoukou

I. Objectives of the project:

The main objective of the project is the estimation of the radiation risk resulting from the operation of nuclear power plants, in the framework of the extensive non-uniformity of population and environment in Greece, putting emphasis at the same time on adopting appropriate population distribution criteria. This risk will be also set in perspective by comparing it to the corresponding risks of alternate energy sources and other technological activities in Greece, focusing on developing an index of harm common to all energy production and other industrial facilities.

II. Objectives for the reporting period:

(a) Continuation of the methodology development for assessing optimum emergency response policies, exploring certain computational problems created by existing statistical dependencies among variables in various steps of the risk calculations,

(b) Assessment of the risk from large nuclear installations operating within or outside Greece, including nuclear powered ships, and

(c) Continuation and improvement of the methodology developed for nuclear power plant siting near large population centres.

III. Progress achieved:

1. Methodology

A procedure for the optimization of the short-term emergency response in the event of a nuclear accident has been developed. The optimization is based on the principles of multiobjective optimization. Three criteria are being used, namely acute fatalities, latent fatalities, and cost of the emergency response policy . The optimization procedure defines the"efficient frontier" that is the emergency response policies that are not dominated by any other response policy in the three criteria mentioned above.

The assessment of reactor accident consequences was performed by employing CRAC.CAEC, the NRCPS "Demokritos" version of the CRAC2 code.

2. Results

A "dynamic programming" procedure for the establishment of the"efficiency frontier" has been developed and implemented in a computer code. This procedure establishes which alternatives (out of all possible emergency policies) can not be excluded on the basis of the expected values of acute fa - talities, latent fatalities, and cost associated with the implementation of the emergency policy.

The set of the "non-dominated" policies can provide useful insights on the range of available policies and the corresponding consequences. Further selection among the policies requires value trade offs among the attributes.

The development procedure assumes that one emergency policy is established on the basis of the available information about possible weather conditions and type of accident.

The methodology developed for selecting a nuclear power plant site near a large population center has been improved and used in a series of specific and generic nature studies. The methodology can be utilized as a siting decision aid tool when deciding on the acceptability of sites, or when sites which are otherwise equivalent, are compared.

The consequence analysis of accidents of nuclear powered ships to the population residing in large ports indicated that the consequences of a severe accident could be substantial.

The analysis of large scale nuclear accidents with transboundary consequences leads to the conclusion that the magnitudes of the potential effects from the severe releases that were adopted indicate that multinational emergency planning for nuclear installations, may be required even when nuclear power stations do not lie in proximity to national borders.

3. Discussion

The optimization of emergency response planning is being achieved in the sense of excluding the dominated policies. An emergency response policy is dominated by another if the former results in higher expected acute fatalities, latent fatalities, and cost. The developed procedure defines all policies that are not dominated by any other policy out of the set of all possible policies. An emergency response policy defines the protective action for each spatial point around the plant. Six protective actions have been included:

(1) evacuation,
(2) shielding in houses for one day,
(3) shielding in houses for seven days,
(4) shielding in large buildings for one day,
(5) shielding in large buildings for seven days, and
(6) continuation of normal activity.

The emergency response policy is established in the face of uncertainty about the weather conditions prevailing at the time of the accident, as well as the type of accident. This uncertainty is quantified on one hand by existing meteorological data, and on the other by probabilistic risk assessment models.

The present version of the developed computer code is basedon CRAC2. The procedure and the developed code is however very general and can accept results of any other consequence code. Presently the methodology is developed assuming uniform population distribution around the plant and radial evacuation direction.

The semi-probabilistic site selection approach that was formulated, has three advantages. It can be used together with other approaches, thus strenthening the final decision. It takes into account the social risk involved and can thus be considered a very desirable supplement to the more common site selection approaches used up to now, which mainly satisfy individual risk criteria. Finally, it is very simple and may ease public fears to some extent by being conservative in certain aspects.

The policy of avoiding port entry of a nuclear powered ship in a large port, would not be different in essence from the siting policy followed in land based nuclear power stations, where proximity to large population centers is avoided.

IV. Objectives for the next reporting period:

(a) Continuation of the methodology development for the assessment of optimum emergency response policy (ERP), exploring the possibility of establishing the ERP after obtaining more information about the nature of the accident (type of release and prevailing weather conditions),
(b) Formulation of isorisk curves for the whole country, in respect to nuclear power plant siting, and
(c) Continuation of the methodology development for nuclear power plant siting near large population centers.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

1. I. Papazoglou: Optimization of Emergency Protective Planning for Major Industrial Accidents, Paper presented at the First Conference of the European Section of the "Society for Risk Analysis", IIASA, Luxemburg, Austria, Nov. 10-11, 1988.

2. J.G. Kollas: Risk Comparisons as Decision Aid Tool for Siting Nuclear Power Plants near Large Population Centers, Proc. Intl. Conf. "Radiation Protection in Nuclear Energy", Sydney, Australia, April 18-22, 1988, Vol. I, p. 413-421.

3. J.G. Kollas: Radiation Risks of Large Scale Nuclear Accidents - A Case Study, Proc. 7th Intl. Congr. of IRPA "Radiation Protection Practice", Sydney, Australia, April 10-17, 1988, vol. II, p. 995-98.

4. J.G. Kollas, and E. Daoukou: The Risk of Nuclear Powered Ships at Large Ports, Proc. of IASTED Intl. Symp. "Modelling, Identification and Control", Grindelwald, Switzerland, Feb. 17-19, 1988, p. 118-21.

Title of the project no.: 2

Wind Flow and Dispersion Aspects of Radiation Risk Assessment

Head(s) of project: J.G. Bartzis

Scientific staff: Antoniadis, J., Catsaros N., Konte K., Megaritou A., Varvayanni M.

I. Objectives of the project:

Transient three-dimensional atmospheric dispersion capability under any atmospheric stability taking into consideration realistic topography including among others, mountains, hills, surface water and islands incorporated into computer code ADREA. The code is intended to be on one hand a "production" code for Environment Institutions and on the other hand a "module" useful to accident consequence analysis.
The study of the flow fields of the lower atmosphere in the Greek territory in the framework of specifying boundary conditions and input data for the atmospheric modelling and the accident consequence analysis in general.

II. Objectives for the reporting period:

- Further work on the 2-D reference problem: diurnal wind flow and dispersion calculations in a terrain simulating the area consisting of the Agean sea coast, Spata plateau (10 km wide), Hymettus mountain (1024 m), Athens basin with emphasis on sea breeze penetration into the city.
- Air/ground and air/sea interaction modelling
- Further verification studies.

III. Progress achieved:

1. Methodology

The analytical work has been performed with the ADREA-I code which is under development in NRCPS "Demokritos" mainly within the framework of this project. ADREA-I is intended to be aplicable to any terrain (complex or not) under any stability atmospheric conditions. At present the mass, momentum energy (temperature), pollutant and turbulent kinetic energy dissipation concervation equations have been already utilized.
During this period the emphasis has been given in further verification of the turbulent diffusion modelling in improving ADREA-I code efficiency and in establishing capability for complex terrain wind flow and dispersion analysis in presence of sea breeze effects, under calm conditions. In addition modifications of the finite difference equations have been performed to improve the efficiency and accuracy of the code.

2. Results

The combined effect of the sea breeze circulations developed under calm conditions at each side of a surrounded by sea mountain, on the contamination patterns, due to a point source located at one side, as well as on the degree of the contaminants penetration into the opposite side has been studied. A two dimensional, 1000 m high mountain range surrounded by sea and respresenting an idealized west-east cross section of the Athens basin has been used for the analysis (3).
Within the framework of performing verification studies of the ADREA-I code, the code was tested against real experimental data, focusing to the Australian Wangera experiment (May, July and August 1976), in order to provide a detailed prediction of the boundary layer structure (4).
In view of air/ground interaction modelling a review has been carried out. In this review methodologies used by investigators to represent air/ground interaction in mesoscale models are analytically discussed (5).

3. Discussion

The sudy of the effects of the sea breeze circulations, developed at each side of a surrounded by sea mountain under calm and stable conditions on the contamination patterns due to a point source located at one side, as well as the degree of penetration to the other side, shows that the inland advancing currents are reinforced by the anabatic winds, resulting to a strong upward motion around and over the mountain top and a quite high boundary layer. The contaminants are mainly advanced in high altitudes. In the opposite side, the upper layers are mainly affected, while the lower altitudes are relatively unaffected due mainly to the mountain presence (3). The results obtained underline on one hand the need for advanced models, such as those used in ADREA-I, to describe complex situations and

on the other hand the difficulty of simple models to cope with such problems (3).
The ADREA-I code, tested against the Wangara field experimental data, provided temperatures, wind velocities, boundary layer height and specific humidity predictions, which compared with the observations can be considered as satisfactory (4).
The air/ground interaction modelling review shows that the majority of the theoretical studies existing in the open literature, either utilize a periodic heating function to prescribe the ground surface temperature, or use the surface Heat Energy budget equation which permites feedbacks between the ground temperature and the atmosphere. The model selected to be incorporated in the ADREA-I code is under examination (5).

IV. Objectives for the next reporting period:

- Further work on complex terrain/sea interaction
- Air/ground interaction modelling
- ADREA-I code documentation
- Investigation of new numerical schemes

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

D. Assimakopoulos, C. Karras, D. Pissimanis, V. Notaridou
Meteorology Laboratory
University of Athens

VI. Publications:

1. Bartzis, J.G., Turbulent Diffusion Modelling for Wind-flow and Dispersion Analysis, Workshop on recent Advances in Reactor Accident Consequence Assessment, Rome, Jan. 27-29 , 1988.

2. Varvayanni M., Bartzis J.G. and Catsaros N., A Theoretical Investigation of Large Natural Barrier Effects on Sea Breeze and Contamination Patterns. Workshop on recent advances in reactor accident consequences assessment Rome, Jan. 27-29, 1988.

3. Bartzis, J.G., Varvayanni, M., Cornelios N., An Analytical Study of Natural Barrier Effects on Sea Breeze and Dispersion. Meteorology and Atmospheric Dispersion in Coastal Area, Riso Nat. Lab., Denmark, Oct. 25-27, 1988.

4. Varvayanni, M., Bartzis J.G., Catsaros, N., ADREA-I Verification Studies Based on Wangara Experiment, To be published.

5. Varvayanni, M., Bartzis, J.G., Air/Ground Interaction Modelling Review. To be published.

Title of the project no.: 3

"Investigation of Enhanced Natural and Artificial Environmental Radioactivity in Greece"

Head(s) of project: Dr. P. Kritidis

Scientific staff: Dr. E. Papanicolaou
Mrs.H Florou
Dr. S. Synetos
Dr. P. Panaiotidis
Mrs.C. Chaloulou

I. Objectives of the project:

1. Investigation of areas with elevated concentrations of natural radioactivity in air and its sources. This includes Greek radon spas, well water supplies, building materials and indoor air. Estimation of occupational and population doses.
2. Investigation of the behaviour of long-lived radionuclides into coastal marine ecosystems, including their uptake and retention by living organisms and their transfer through the various trophic levels.

II. Objectives for the reporting period:

1. Ivestigation of certain regions in Northern Greece and Aegean Sea with enhanced concentrations of U-238 and Th-232 series radionuclides.
2. Natural radioactivity maping of the Greek soils by use of the samples collected for caesium deposition maping.
3. A more detailed study of the impact of the Chernobyl accident on the fresh water ecosystems, with emphasis to lake and estuary environments.
4. Investigation of the unique coastal regions in Ikaria and Evoia islands where significant amounts of spa waters with enhanced content of Ra-226 are released in the sea.

III. Progress achieved:

1. Methodology. The methods used during 1988 include:

- High-resolution gamma spectrometry with computer analysis.
- Two total-alpha counting methods for determination of radon daughters in air.
- Lucas cell total alpha counting for determination of Rn-222 and Rn-220 in air and liquid samples.
- Car-born scintillometry.

2. Results

2.1. Collection of sediments has been implemented in a dense sampling network around the Milos island as well as inside its gulf. The analysis showed that the levels of natural radioactivity in the Milos sediments are enhanced in comparison with those observed in other areas of the Greek peninsula.

2.2. A large number of Milos ore samples has been collected and currently analysed. The concentrations of natural radionuclides appear to be enhanced, with indicative values as follows:

Nucl.	min.	max.	aver.	(Bq/kg)
Ra-226	41	96	68	
Th-232	48	121	80	
K-40	184	1490	850	

The analysis has not been completed yet. A CBS study of Milos island has been carried out as well. The maximum exposure rates observed (25 μR/ h) are in accordance with gamma-spectrometry findings.

2.3. The analysis of the natural radioactivity in Greek soil samples continued during 1988. Of 130 samples analysed the indicative values of the activity concentrations are:

Nucl.	min.	max.	aver.	(Bq/kg)
Ra-226	7	217	38	
Th-232	5	125	45	
K-40	26	1480	520	

This work intends to create a valuable part of a Greek environmental radioactivity databank.

2.4. The investigation of the delayed impact of the Chernobyl accident on the Greek environment has been continued:

- Analysis of paired soil/plant samples (1988 harvest) has been carried out to determine the soil-to-plant transfer factors for caesium and, more generally, the 2nd-year deposition-to-plant transfer factors. These factors appear to lay on the lowest side of the ranges reported in Europe and they are of definitely low radiological importance.

- A second collection of lake fish has been carried out according to the sampling network established after the Chernobyl accident. The samples are still analyzed.
- The routine measurements of the total-beta activity in air and in surface waters have been statistically analysed and the results compared with those refering to the period prior to the Chernobyl accident. A slide but statistically significant increase has been observed in both cases. Especially in the case of air, the increase can be fully assigned to the re-suspension effect.

2.5. Certain coastal regions of Ikaria island have been investigated in order to determine the impact of the continuous release of spa waters of enhanced Ra-226 content to the local marine biota. The samples are still analyzed.

3. Discussion

The enhanced levels of natural radioactivity in Milos island area are associated with the volvanic origin of the region. The investigation of this area intends to provide a radiological example of enhanced population exposure to the natural radioactivity, useful for various comparisons with cases of exposure to radiations of artificial origin.

The available results of the natural radioactivity maping of the Greek soils indicate the existence of other regions of enhanced concentrations of U-234 and Th-232 series nuclides, located, generally, in the Northern part of the country.

The delayed impact of the Chernobyl accident on the Greek environment seems to be of minor radiological importance if compared with the first year impact.

IV. Objectives for the next reporting period:

1. Investigations of regions of enhanced natural radioactivity in Northern and Central Greece and in the Aegean Sea.
2. Continuation of the natural radioactivity mapping of the Greek soils.
3. Continuation of the studies on the late impact of the Chernobyl accident on the Greek environment.
4. Introduction of the track-etch detection technology in the studies of radon levels in Greek dwellings.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1. The group of Marine Radioecology, National Institute for Marine Research, Aghios Cosmas, Helliniko, Attiki (Mrs. H. Florou, Dr. P. Panaiotidis).
2. The group of Soil Research, NRCPS "DEMOKRITOS", Aghia Paraskevi Attikis (Dr. E. Pananikolaou).

VI. Publications:

1. P. Kritidis and M. Probonas, The Greek radon spas: hot spots of natural radioactivity in the Mediterranean area", Intern. Conf. on Environm. Radioact. in the Mediter. Area, Barcelona (Spain), 10-13 May 1988.
2. H. Florou and P. Kritidis, "Enhanced natural radioactivity in some areas of Aegean achipelago", Intern. Conf. on Environm. Radioact. in the Mediter. Area, Barcelona (Spain), 10-13 May 1988.
3. E. Papanicolaou and P. Kritidis, "Contamination of the agricultural land of Greece with radioactive caesium and its effect on the growing crops", Intern. Conf. on Environm. Radioact. in the Mediter. Areas, Barcelona (Spain),10-13 May 1988.
4. P. Kritidis, "The lessons of the Chernobyl accident in regard to the environmental radioactivity control", Scient. Conference of the Balkan Academy on Environm. Protection, Varna (Bulgaria), 19-23 Sept. 1988.
5. P. Kritidis, "Radioactive balneological spas and radiation protection principles", II Nation. Congress for the Thermometallic Waters, Thessaloniki (Greece), 7-9 Oct. 1988.

6.P. Kritidis, H. Florou and S. Synetos: "The contribution of fish consumption to the dose received by the Greek population due to the Chernobyl accident", XXXI Congr. of the Internat. Committee for Scient. Explor. of the Mediterranean Sea, Athens (Greece), 17-22 Oct. 1988. (also "Thalassografica", in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-115-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. G. Madelaine
IPSN
CEA-CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Telephone number: (1) 654.71.36

Title of the research contract:

Characterization of radon daughters and carcinogenesis.

List of projects:

1. Characterization of radon daughters and carcinogenesis.

Title of the project no.:

Caractérisation de la granulométrie et des propriétés électriques des descendants du radon en relation avec l'étude de la carcinogenèse pulmonaire chez le rat

Head(s) of project:

Dr. G. MADELAINE, chef des Laboratoires d'Etudes des Pollutions Atmosphériques, DPT/SPIN, Centre d'Etudes Nucléaires de Fontenay-aux-Roses, BP 6, 92265 FONTENAY AUX ROSES CEDEX

Scientific staff:

Dr. D. BOULAUD, chef du Laboratoire de Physique et Métrologie des Aérosols, DPT/SPIN/LEPA, Centre d'Etudes Nucléaires de Fontenay-aux-Roses, BP 6, 92265 FONTENAY AUX ROSES CEDEX

I. Objectives of the project:

Caractérisation de la répartition en dimension et de l'état de charge électrique des descendants du radon inhalés par le rat dans la chambre d'exposition du CEA à Razès, Division Minière de la COGEMA. Ces paramètres influent sur la dose reçue par les cellules des différents compartiments pulmonaires.

II. Objectives for the reporting period:

Mesures systématiques de la granulométrie des descendants du radon fixés sur les aérosols. Comportement des impacteurs vis-à-vis des particules submicroniques.

III. Progress achieved:

Le programme des travaux de 1988 a porté sur des mesures systématiques de la répartition de la radioactivité des descendants du radon dans la chambre d'exposition en présence ou non de rats pour tenter de mettre en évidence une évolution spatio-temporelle de cette répartition.

On rappelle que cette répartion de l'activité est obtenue avec le Spectromètre Diffusionnel et Inertiel (SDI 2000) mis au point à cet effet. L'association à l'étage de diffusion d'un impacteur en cascade permet d'obtenir des informations sur la radioactivité fixée sur les particules de dimension supérieure à 0,4 µm environ. Le dispositif a été utilisé en interposant en amont des grilles destinées à csptée la fraction libre pouvant être retenue par les étages d'impaction et venir ainsi modifier la granulométrie des "aérosols radioactifs".

Les séries de mesure effectuées ont donné, pour des conditions identiques, des résultats reproductibles dans la majorité des cas.

Le diamètre moyen en activité de l'aérosol dans l'enceinte d'exposition a été trouvé à 0,26 µm avec un σg de 2,9.

En présence de rats (50 rats répartis dans 10 cages) ce diamètre moyen passe à 0,35 µm avec un σg de 3.

Il semblerait que la présence d'animaux augmente légèrement la dimension de l'aérosol.

En fonction de la concentration et de la granulométrie des aérosols présents dans l'enceinte, certaines anomalies sont apparues et la valeur élevée du σg trouvé nous a conduit à préciser le comportement des étages inertiels vis-à-vis des aérosols submicroniques. On souligne que si ce piégeage a peu d'importance dans la détermination d'une granulométrie en masse, il peut par contre modifier sensiblement une granulométrie en nombre (cas des descendants solides des gaz radioactifs naturels ou artificiels).

Un étalonnage avec des aérosols monodispersés a pu montrer que, dans la gamme 0,05-0,1 µm, environ 10 % des particules étsient réparties sur les différents étages de l'impacteur.

Un autre problème est apparu concernant la nature de la surface de collection et son influence sur la réponse granulométrique du dispositif SDI 2000. Trois surfaces d'impaction ont été étudiées, plaques de verre, métalliques imprégnées de graisse ou filtre fibreux et membranes microporeuses. Il est apparu que la collection des aérosols par un média filtrant entraînait, par filtration tangentielle et pénétration du jet à travers une très faible épaisseur du filtre, une captation des particules fines entraînant un élargissement du spectre granulométrique (augmentation du σg).

En conclusion, les résultsts déjà obtenus, ainsi que les améliorations apportées au Spectromètre Diffusionnel et Inertiel par un étalonnage précis devront nous permettre d'obtenir une plus grande précision sur l'étude de la granulométrie des descendants du radon dans diverses atmosphères. L'obtention d'une telle granulométrie avec uniquement des dispositifs diffusionnels utilisés dans la majorité des cas, pouvant être insuffisant pour couvrir la totalité de la gamme de dimension.

IV. Objectives for the next reporting period:

Application à des mesures de routine du Spectromètre Diffusionnel et Inertiel : contrôle de la granulométrie dans la chambre d'exposition, dans l'environnement et à l'intérieur des locaux.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

M. DIOURI
Contribution à l'étude du comportement aérodynamique des aérosols.
Mise au point d'un Spectromètre Diffusionnel et Inertiel
Rapport CEA-R-5412, 1987

M. DIOURI, D. BOULAUD, G. MADELAINE
A new diffusional and inertial spectrometer (SDI 2000)
AAAR Meeting, Seattle, USA, september 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

University College Dublin
Belfield
IRL- Dublin 4

Contract no.: BI6-P-117-IRL

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.P. McLaughlin
Physics Department
University College Dublin
Belfield
IRL- Dublin 4

Telephone number: 69.32.44

Title of the research contract:

Assessment of the population dose indoors from natural radiation in Ireland with particular emphasis on radon daughter properties and behaviour.

List of projects:

1. Assessment of the population dose indoors from natural radiation in Ireland.
2. Investigations on the plateout velocities and removal rates of airborne Radon daughters.

Title of the project no.: (1) Assessment of the population dose indoors from natural radiation in Ireland.

Head(s) of project: Dr. J.P. McLaughlin

Scientific staff: P. Wasiolek.

I. Objectives of the project:

(i) To carry out a national survey of radon concentrations in Irish dwellings

(ii) To analyse the survey data to determine the effective dose equivalent size distribution arising from the inhalation of radon daughters by the Irish population.

(iii) To identify factors such as soil/geological characteristics, building practices etc. which are most likely to give rise to elevated indoor air radon concentrations and associated lung doses.

II. Objectives for the reporting period:

(1) Completion of random phase of national survey of indoor radon and analysis of data.

(2) Commencement of field intercomparisons of passive radon detectors in French, Irish and Italian dwellings.

III. Progress achieved: National Indoor radon survey

By the end of 1988 indoor radon had been measured in over 1400 Irish dwellings. Of this total number 1259 were selected in a random fashion using the national electoral register as a sampling frame. Passive closed alpha track (CR-39 radon detectors were used in the survey which used the postal services for detector distribution and recovery. The indoor radon levels determined for the random set were found to be log-normally distributed with a median value of 34 Bq/m^3 and a geometric standard deviation $\sigma_g = 2.5$. About 1.6% of the dwellings investigated had indoor radon concentrations in excess of 400 Bq/m^3. Each participating household was sent a questionnaire to complete consisting of 27 questions relating to characteristics of both the house and its occupants. A total of 943 such questionnaires were returned completed. The information contained in these was first analysed to determine if the households which participated were representative of the Irish population. Various statistical tests were applied to the questionnaire data on information relating to occupant age and social category distributions. The overall result of this analysis shows that the participating households were indeed quite representative of the present Irish population. From analysis of the survey data no strong correlations with indoor radon have been found either with the physical characteristics of the houses or the geological properties of their locations. In this latter regard the assistance of the Geological Survey of Ireland was obtained. There are however indications that some form of physical geological controls such as faults, soil permeability etc may play a more important role in determining indoor radon in Ireland than the radium-226 content of surface rocks or soils. It should also be noted that precise plotting of investigated houses on geological maps was difficult because all the radon measurements were made using postal addresses which do not generally allow for map plotting of sufficient accuracy for geological interpretation. This difficulty will be overcome in 1989 with a regional indoor radon survey in the west of Ireland where each house will be visited and it is intended to study soil characteristics at each site. The area now selected for this study (approx. 5000 km^2) is principally in the western counties of Clare, Galway and Mayo where both high indoor radon levels have been detected in this contract work and where an independent study (Dr. I.R. McAulay, Trinity College Dublin) has found high levels of

radium-226 in soils. Assistance in obtaining volunteer households was requested and obtained from health officials in this area.

INTERCOMPARISONS OF PASSIVE RADON DETECTORS IN FRENCH, IRISH AND ITALIAN DWELLINGS. Collaboration between University College Dublin, CEA (Fontenay aux Roses, France) and ENEA (Roma) has resulted in the placement during October 1988 of a total of 270 passive alpha track radon detectors distributed between the countries of the participating laboratories. Three dwellings were chosen in each country and in each dwelling ten detectors from each laboratory were exposed. The dwellings were chosen to approximately represent average (20 to 50 Bq/m^3) and intermediate (200 Bq/m^3) and elevated (400 Bq/m^3) radon exposure situations. Two types of closed detectors (I,IRL) and one open face type (FR) were used. Procedures and protocols (such as the use of heat sealed radon impermeable bags during the transport of the detectors) were established to minimise background effects and to ensure a comparable and good control of the exposures in the three countries. The exposures are scheduled to terminate at the end of January 1989. Apart from its scientific value it is hoped that this exercise may compliment the established programme of the CEC and the NRPB (UK) in carrying out laboratory intercomparisons. It is also hoped that it may serve as a pilot study from which the experience gained may help to initiate an extended intercomparison of this type involving more European laboratories.

IV. Objectives for the next reporting period:

It is intended to complete the analysis of the indoor radon survey data. The regional survey in the west of Ireland will be initiated in collaboration with other Irish universities and State laboratories.
The intercomparison of detectors in France, Ireland and Italy will be completed and its findings will be published.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

(1) Dr. I.R. Mc Aulay, Department of Pure and Applied Physics, Trinity College Dublin Ireland.

(2) Dr. A. Rannou, Commissariat a l'Energie Atomique, IPSN-DPS-SHR-SEAPS-BP No.6 - Fontenay aux Roses, France.

(3) Drs L. Tommasino and G.C. Torri
DISP, ENEA, Roma, Italia.

VI. Publications:

None during the reporting period.

Title of the project no.: (2) Investigations on the plateout velocities and removal rates of airborne radon daughters.

Head(s) of project: Dr. J.P. McLaughlin.

Scientific staff: P. Wasiolek.

I. Objectives of the project: It is intended to make a study of radon daughter behaviour in an experimental room. Accurate determinations will be made of such important properties of the radon daughters as their plateout velocities and removal rates under various aerosol loading and ventilation conditions. With the aid of devices such as electro-filters it is intended to investigate the feasibility of reducing the potential alpha energy concentration and consequently the expected lung dose due to radon daughters in a room.

II. Objectives for the reporting period: A study of the effectiveness of reducing the PAEC (potential alpha energy concentration) of radon daughters in room air by means of the combined effects of electric fields and unipolar ion production.

III. Progress achieved: A series of investigations into the reduction of the PAEC (potential alpha energy concentration) of airborne radon decay products in a 44 m^3 experimental room were carried out at radon gas concentrations up to 10000 Bq/m^3. These reductions were acheived by establishing electric fields and by generating unipolar ions in the room air. Under the most extreme conditions tested using a positive 30 kV potential on a steel needle and an air ion current of 2.5 μA a reduction of 94% in the PAEC was acheived. At the lower potential of + 5 kV the PAEC reduction is about 50%. A summary of the experimental results are shown in Figure 1. The combined effect of positive potential and positive ions was found to be more effective than when negative polarity is used. It is still not clear whether the substantial reductions in PAEC acheivable by these techniques necessarily results in a useful reduction in dose to an exposed individual. Current lung dose models ascribe a much greater dose per unit exposure to the unattached fraction of radon daughters than to the attached fraction. As the field-ion treatment increases the relative fraction of unattached daughters this suggests that the benefit in reducing the PAEC may be somewhat offset by the increase in unattached fraction. For the air treatments investigated the application of recent lung dose models suggests that the percentage reduction in lung dose is about half the percentage reduction in PAEC acheived. The overall behaviour of the radon daughters in the experimental room with and without the use of fields and ions was further studied by investigating their deposition or plateout behaviour onto room surfaces. This was measured using pieces of alpha track plastic detectors (LR-115 and CR-39) which were mounted on the various room surfaces. The LR-115 plastic was used to give a measure of the total airborne alpha activity in the room and the CR-39 to give in addition a measure of the plateout activity distribution on room surfaces. While this approach was semi-quantitative the effectiveness of the field - ion air treatments in causing enhanced plateout was well verified. Using the plastic detectors as alpha detectors on room surfaces it was shown possible to modify the electric field configurations using metal plates to cause preferential deposition onto such surfaces as ceilings. It is planned in the next phase of the work to improve the plateout measurement technique so that the deposition rate constants for the radon daughters may be accurately measured.

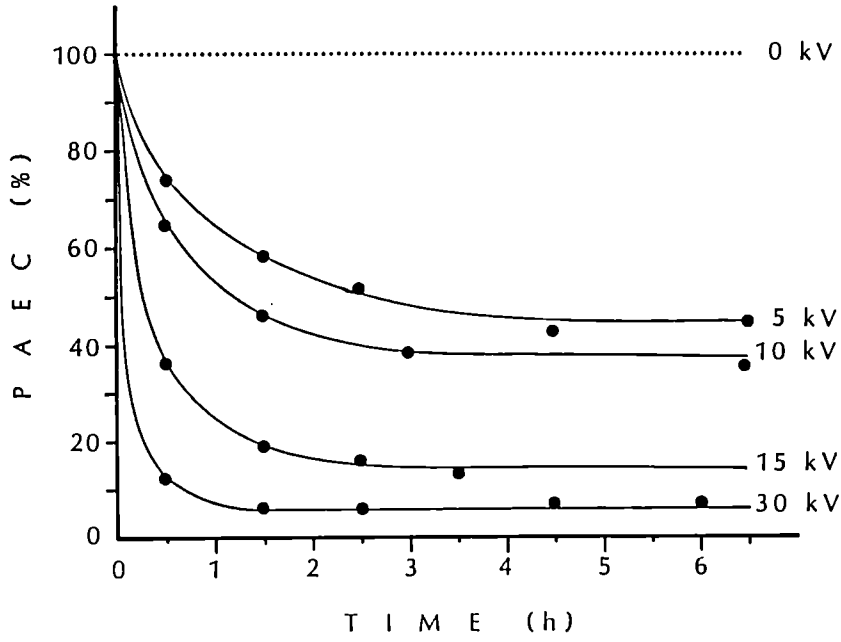


Fig. 1 PAEC percentage reductions using combined positive ions and field.

IV. Objectives for the next reporting period: It is planned to make quantitative measurements of the deposition rate constants of the radon daughters within the experimental room with and without field - ion treatments. This will involve the development of a new technique of radon daughter plateout measurement. Preliminary tests have indicated that it may be possible using this technique to measure the plateout properties of the individual radon daughters.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Niels Jonassen, Technical University of Denmark
Laboratory of Applied Physics 1,
Lyngby, Denmark.

VI. Publications:

McLaughlin, J.P. and Wasiolek, P. "Experimental Room Studies on the Reduction of Radon Progeny Concentrations using Unipolar Ions and Electric Fields" presented at The U.S. EPA 1988 Symposium on Radon and Radon Reduction Technology Denver, Colorado. October 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Risø National Laboratory
P.O. Box 49
DK-4000 Roskilde

Contract no.: BI6-F-296-DK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. T. Mikkelsen
Meteorology Section
Risø National Laboratory
P.O. Box 49
DK-4000 Roskilde

Telephone number: (45) 2371212

Title of the research contract:

Validation experiments for near-site region atmospheric dispersion models.

List of projects:

1. Validation experiments for near-site region atmospheric dispersion models.

Title of the project no.:

Validation experiments for near-site region atmospheric dispersion models.

Head(s) of project:

Dr. T. Mikkelsen
Meteorology and Wind Energy Department
Risø National Laboratory
DK-4000 Roskilde, Denmark

Scientific staff:

Mr. Leif Kristensen
Mr. S. Thykier-Nielsen
Dr. H.L. Pécseli

I. Objectives of the project:

From full-scale diffusion experiments to establish reference data sets of instantaneous crosswind concentration profiles, from which uncertainties in atmospheric dispersion models, used in probabilistic accident consequence codes, can be assessed.

II. Objectives for the reporting period:

This first year of work (1988) has been devoted to preparation and field testing of a new Mini-LIDAR system to measure instantaneous concentration profiles. In accordance with this, the LIDAR system should emerge to be operational and calibrated, and its practical limitations should be explored. Pilot tests with artificially generated aerosol plumes should be undertaken.

1. Methodology

Efforts to prepare equipment and experimental procedures culminated in a full-scale field test performed at Meppen Proving Grounds (FRG) in October. Here was brought together:

1. Our newly assembled Mini-LIDAR system including a laser, telescope, high-speed detector and amplifier, transient recorder, and PC-controlled data storage and monitoring facilities.
2. An artificial aerosol generator (white smoke).
3. Boundary-layer mean wind and turbulence instruments (sonic-anemometers).
4. An aerosol particle-counter and size-distribution measurement system.

2. Results

A total of 16 field tests, some of which continued over several hours, yielded some 10,000 instantaneous crosswind backscatter profiles measured by the LIDAR system. During the exsperiments we encountered different atmospheric stability situations and measured at various downwind distances. Crosswind concentration profiles were subsequently calculated from the measured backscatter profiles measured (raw data) by quality assurance and calibration involving: (1) range and extinction correction, (2) background and noise reduction.

The preliminary field tests in 1988 have provided us with the following experience with our LIDAR system:

1. We have accomplished the main LIDAR design-criterion set for measuring in-plume concentration profiles with high spacial resolution (1.5 m) and high repetition rate (3s).
2. The dynamical range of the system has proven satisfactory; however, additional detector amplification is required to obtain profiles beyond a $\sim$ 1-km downwind range.
3. Computer programs for background and noise reduction works, but can be improved. On-line algorithms for range and extension losses must be automated.
4. An attempt to absolute-calibrate the LIDAR system must await improvements in the points (2) ande (3) above.

Also in the reporting period, algorithms and procedures have been developed for the *subsequent* statistical analysis of the concentration profiles measured.

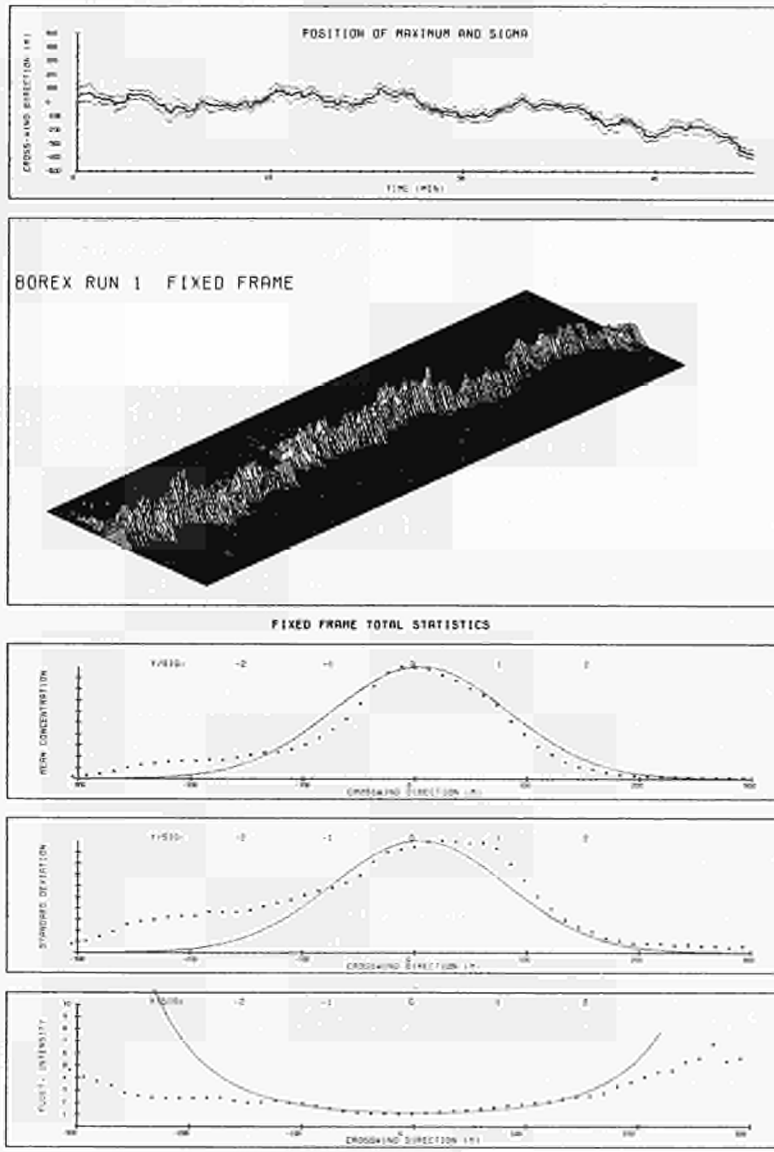
Data reduction algorithms, applicable for producing individual "validation reference data sets" have been prepared accordingly to calculate crosswind profiles of:

1. mean and mean-square concentrations,
2. plume meander,
3. instantaneous and time-averaged (variable) dispersion coefficients,
4. intermittence and concentration-intensity measures.

Figure 1 shows an example of results obtained at 1-km downwind distance from a one-hour experiment performed under near-neutral atmospheric stability.

3. Discussion

The pilot field test indicate that upon minor corrections and improvements, we are prepared for the measurement campaigns already scheduled for 1989.



Result from the pilot-validation experiment BOREX RUN 1: (1) plume-meander and instantaneous plume width); (2) time sequence of instantaneously measured crosswind concentration profiles; (3) measured mean-concentration profile (·), Gauss fit (full line); (4) measured concentration-fluctuation standard deviation profile (·), Gauss fit (full line); (5) measured intensity profile (standard deviation/mean concentration) (·), model by Wilson et al. (1985)(full line).

Fig.1

IV. Objectives for the next reporting period:

After minor improvements of the LIDAR system and its data processing programs to conduct measurement campaigns already scheduled, to process and quality assure thereby obtained backscatter profiles into concentration profiles, which in turn will be statistically analysed and classified together with simultaneously measured meteorological data in a series of 'validation' experiments.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Aerospace Research Establishment, DFVLR (FRG) C. Verner, H Herrmann
Amt für Wehrgeophysik, GMGO, Traben-Trarbach (FRG) H.Weber, W. aufm Kampe
IBS Vertriebs-GmbH, Grafrath (FRG) B.Scholtz
C.E.N., Mol (Belgium) A. Sohier
UNI-MAINZ (FRG) S. Borrmann

VI. Publications:

Weber, H., W. aufmKampe, and T. Mikkelsen (1988). Concentration fluctuations measured in the atmospheric surface layer. In: Eighth Symposium on Turbulence and Diffusion (Published by the American Meteorological Society, Boston, MA, USA).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-121-F

Centre de Développement des Etudes
et Applications en Hygiène
et Sécurité
18, Avenue Fontcouverte
F-84000 Avignon

Head(s) of research team(s) [name(s) and address(es)]:

Mr. G. Morlat
Serv. d'Etudes Gén. de Protec.
C.E.D.H.Y.S
118 rue de la Tombe Issoire
F-75014 Paris

M. F. Anguenot
IPSN.DPS/SEGP
B.P. n° 6
F-92265 Fontenay-aux-Roses

Telephone number:

Title of the research contract:

Comparative risk evaluation on a regional scale.

List of projects:

1. Comparative risk evaluation on a regional scale.

Title of the project no.: 1

Comparative risk evaluation on a regional scale of energy production and utilization and other main industrial activities with respect to their consequences for workers, population and environment, including the assessment of catastrophic events.

Head(s) of project:

Mr G. Morlat
Serv. Etudes Gén. Protec.
C.E.D.H.Y.S.
118 rue de la Tombe Issoire
75014 Paris

Mr F. Anguenot
IPSN.DPS/SEGP
BP n°6
F-92265 Fontenay aux Roses
Cedex

Scientific staff:

5 personnes

I. Objectives of the project:

- L'objectif de cette étude est la mise en perspective des différentes nuisances (radiologiques ou non) auxquelles est soumise une population régionale (Sud-Est de la France), que ces nuisances soient d'origine industrielle, domestique, naturelle voire agricole.
- La gestion des nuisances ne peut permettre de privilégier, à priori, l'une ou l'autre des sources d'exposition. Il faut donc, au préalable, en effectuer l'évaluation dans un contexte réel.
- En complément à cette analyse des nuisances une reflexion est menée sur la possibilité d'agrégation, par le décideur, des évaluations du risque obtenues en fonctionnement normal des installations à celles estimées à la suite d'un accident.

II. Objectives for the reporting period:

Les trois directions de recherche pour 1988 ont été, suite à la constitution des banques de données réalisées en 1987 :
- L'évaluation des expositions, pour le public, résultant du chauffage résidentiel et tertiaire.
- La détermination des facteurs d'émission des composés organiques volatils (COV) de différentes familles de produits à usage domestique avec mise au point d'un protocole expérimental de mesure selon les différents conditionnements rencontrés (solide, pâte, liquide, aérosol).
- L'analyse du système décisionnel en matière de gestion du risque : comment une étude régionale peut-elle s'intégrer aux circuits décisionnels en matière de gestion du risque, l'exemple français.

III. Progress achieved:

METHODES ET MOYENS

a- Enquêtes au niveau national et départemental

- Population, parc des habitations et des chauffages centraux et indépendants.
- Consommation énergétique par type de combustible.
- Inventaire et consommation des produits à usage domestique (entretien de la maison, bien être corporel, bricolage,...).
- Interviews d'acteurs régionaux par entretiens semi-directifs, analyse de leurs documentations professionnelles et étude de cas en détail.

b- Expérimentation et analyses chimiques

- Détermination expérimentale des paramètres de modélisation d'une chambre d'expérimentation (taux de renouvellement).
- Facteurs d'émission de CO, CO_2, NO_x et SO_2 d'une cuisinière à gaz.
- Facteurs d'émission de composés organiques volatils (COV) et d'HPA par un foyer ouvert à bois.
- Mise au point d'un protocole expérimental d'évaluation des facteurs d'émission de COV par des produits à usage domestique sous différentes formes : solide, pâte, liquide, gaz (bombe aérosol).

RESULTATS ET DISCUSSION

a- Chauffage résidentiel

Les concentrations résultantes, dans les 1133 mailles de 100 km^2 qui couvrent la région étudiée, sont en général plus élevées que celles issues du cycle énergétique fossile pour une même consommation, sauf pour SO_2 et COV (tableau 1). Les hauteurs de rejets plus faibles et les quantités rejetées plus importantes, du fait de l'absence de systèmes de filtration, ainsi que des conditions de combustion en général meilleures dans le secteur industriel que dans le secteur résidentiel expliquent cette différence. L'importance du chauffage résidentiel apparaît ici clairement dans le bilan global des expositions.

	Résidentiel (5200 ktep)			Energétique (2200 ktep)			Tertiaire (1600 ktep)		
	Max.	Min.	C_{50}	Max.	Min.	C_{50}	Max.	Min.	C_{50}
Poussières	5	0,13	0,28	0,4	0,007	0,04	0,5	0,002	0,006
NO_x	11	0,18	0,34	0,7	0,02	0,10	6,1	0,03	0,08
SO_2	14	0,2	0,42	9,5	0,15	1,1	2,1	0,007	0,002
COV	3,5	0,08	0,16	1,1	0,02	0,14	1,3	0,007	0,02
CO_2	7500	150	280	500	10	67	3920	19,9	49,8
CO	95	2,5	5,3	0,16	0,02	0,06	12,4	0,04	0,1

TABLEAU 1 : Maximas (Max), minimas (Min) et médianes (C_{50}) des courbes cumulées des concentrations ($\mu g/m^3$) atmosphériques de polluants émis par le chauffage résidentiel et tertiaire, et, le secteur énergétique (centrales thermiques fossiles et raffineries) du Sud-Est de la France.

b- Chauffage tertiaire

Une démarche similaire à celle utilisée pour le secteur résidentiel montre que, à consommation énergétique égale, les concentrations résultantes du chauffage dans le secteur tertiaire sont plus faibles que celle issues du secteur résidentiel (tableau 1).

c- Pollution à l'intérieur des habitations

*** foyer ouvert à bois**
- La combustion du bois (chêne et charme), surtout pendant la période d'allumage, donne naissance à des produits toxiques : benzène, furfural, phénol, HPA mutagènes. Le rôle des poussières présentes dans les atmosphères étudiées (fumées) comme agent de transfert des HPA a été confirmé.
- Dans le cas où le feu est constitué uniquement de braises, la concentration des composés cités diminue et des produits nouveaux tels que les acides et les esters apparaissent.

*** pratiques culinaires**
Elles ne conduisent pas à la formation significative d'HPA lourds, par contre des aldéhydes saturés et insaturés, des hydrocarbures et le limonène ont été détectés dans l'atmosphère d'une cuisine.

*** produits à usage domestique**
- Une banque de données a été créée rassemblant toutes les informations relatives aux différents produits à usage domestique ainsi que leurs consommations au niveau national et départemental (Sud-Est de la France).
- Les résultats qualitatifs et quantitatifs relatifs aux familles de produits analysés ont montré que les émissions de certains COV peuvent conduire à des expositions aigües élevées (toluène dans les colles) ou chroniques non négligeables (para-dichlorobenzène des blocs WC par exemple).

d- Circuits de l'information et processus de décision

Ce travail fait l'objet d'une thèse qui sera terminée en 1989 :
- Le système de décision en matière de pollution est analysé pour la France : cadre réglementaire, cohérence et principes.
- Le processus conflictuel de décision et le jeu des acteurs sont analysés en s'appuyant sur des observations recueillies sur le terrain.

CONCLUSION

Ces résultats ont le mérite de montrer que les expositions chroniques subies par les populations doivent être prises en compte dans le bilan global des expositions à un moment où la pollution atmosphérique industrielle faisant l'objet de plus en plus de contrôles aurait tendance à diminuer.

IV- OBJECTIVES FOR THE NEXT REPORTING PERIOD

1- La réactualisation des valeurs des émissions des installations du cycle électrogène est en cours et sera terminée pour le rapport de fin de contrat.

2- L'évaluation des facteurs d'émission de COV caractéristiques de produits à usage domestique sera poursuivie et les facteurs d'exposition correspondants dans l'habitat recherchés.

3- L'analyse du risque accidentel (accidents majeurs) sera menée dans le but de son agrégation aux analyses de risque en fonctionnement normal.

4- La gestion du risque dans l'industrie chimique sera comparée à celle du nucléaire et les possibilités de transfert d'un secteur à l'autre seront analysées avec mise en évidence des différences essentielles.

5- Une reflexion générale sur la fiabilité des systèmes sera présentée dans le cadre de la gestion du risque.

6- Enfin, la rédaction du rapport de synthèse des travaux effectués dans le cadre de ce contrat occupera une grande partie de l'année 1989.

V. OTHER RESEARCH GROUP(S) COLLABORATING ACTIVELY ON THIS PROJECT

- Institut de Protection et de Sureté Nucléaire
  Service d'Etudes Générales et de Protection
  B.P. n°6 92265 Fontenay-aux-Roses Cédex
- Institut de Protection et de Sureté Nucléaire
  Service de Protection des Installations Nucléaires
  B.P n 6 92265 Fontenay-aux-Roses Cédex
- Institut de Recherches et de Développements Industriels
  Section d'Etudes et d'Analyses Isotopiques et Nucléaires
  Centre dEtudes Nucléaires de Saclay
  91191 Gif-sur-Yvette Cédex
- Comité Scientifique et Technique de l'Industrie du Chauffage et du Conditionnement d'Air (COSTIC)
  9 rue Lapérouse
  75784 Paris Cédex 16
- Centre d'Etudes et de Recherches Economiques sur l'Energie (CEREN)
  89 rue de Mirosmesnil
  75008 Paris
- Conseils - Recherche - Formation (COREF)
  Tour Chenonceaux-204 rond-point du Pont-de-Sèvres
  92516 Boulogne-Billancourt Cédex
- Laboratoire de Physico-Chimie de l'Atmosphère (Professeur Mouvier)
  Université Paris-VII
  2 place Jussieu
  75251 Paris Cédex 05

VI. PUBLICATIONS

- AIGUEPERSE J., ANGUENOT F., COULON R. A regional approach of risk assessment : the case study of the south-east part of France. International Workshop - ENVRISK 88, Energy and Environment : the european perspective on risk , COME (Italy), May 11-13, 1988.

- COULON R., AIGUEPERSE J., ANGUENOT F. The impact of conventional and nuclear industries on the population: A comparative study of the radioactive and chemical aspects - Report EUR 10557 EN (1988).

- ANGUENOT F., AIGUEPERSE J., BONNEFOUS S., DESPRES A. The contribution of residential heating pollution to environmental nuisances at regional level (Greater Rhône Delta Program - Seminar on applications, perspectives and limitations of comparative risk assessment and risk management, NICE (France), September 11-13, 1988.

- AIGUEPERSE J., ANGUENOT F., PERSON A., LAURENT AM., LOUIS-GAVET MC., FESTY B. The contribution of indoor pollution to the contamination level on a regional basis (Greater Rhône Delta Program - Seminar on applications, perspectives and limitations of comparative risk assessment and risk management, NICE (France), September 11-13, 1988.

- GALLE M. Processus de décision en matière de pollution - Seminar on applications, perspectives and limitations of comparative risk assessment and risk management, NICE (France), September 11-13, 1988.

- AIGUEPERSE J., ANGUENOT F. Evaluation au niveau régional des conséquences sanitaires dues aux rejets atmosphériques de cadmium par les centrales thermiques fossiles du Sud-Est de la France (Programme Grand Delta) - Symposium International de Toxicologie Industrielle, Bordeaux (France) 13-16 décembre 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-118-UK

Head(s) of research team(s) [name(s) and address(es)]:

Mr. M.C. O'Riordan
Radiological Measurement Department
NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: (235) 83.16.00

Title of the research contract:

Impact assessment of artificial and enhanced natural radioactivity in the outdoor and indoor environment.

List of projects:

1. Characterization of radioactive aerosols near a coastal nuclear facility and assessment of their impact.
2. Study of methods for remedying and preventing high radon levels in dwellings.

Title of the project no.:

1

Characterisation of radioactive aerosols near a coastal nuclear facility and assessment of their impact

Head(s) of project:

F A Fry

Scientific staff:

M R Bailey, N J Dodd, G Etherington, N Green

I. Objectives of the project:

To determine the characteristics of the radioactive aerosols created near a coastal nuclear facility and to assess their radiological significance.

II. Objectives for the reporting period:

(a) Construction of a high efficiency air sampler based on the inlet to the WRAC (Wide Range Aerosol Classifier), and investigation of methods for classifying particles collected by it.

(b) Construction and testing of a rotary impactor.

(c) Determination of the collection efficiency of the existing sampler.

III. Progress achieved:

Previous investigations of airborne radionuclides in the environment around the nuclear fuel reprocessing plant at Sellafield in Cumbria, UK, indicate that actinides are the main radioactive constituent of particles with aerodynamic diameters (d_{ae}) greater than 10 μm. Air sampling equipment in current use is unlikely to obtain representative samples of particles with such large aerodynamic diameters. A new type of instrument is therefore required which samples particles representatively up to a d_{ae} of 100 μm over the range of wind speeds encountered in a coastal environment, classifies the particles according to size, has a high sampling rate (several $m^3\ min^{-1}$) and can withstand severe weather conditions. The two types of sampler outlined in II (a) and (b) above were identified as being capable in principle of satisfying these requirements. During this reporting period, effort was concentrated on developing the high-efficiency air sampler because it was considered that problems with weatherproofing and overloading would be greater with the rotary impactor. Work has concentrated initially on the development of an instrument to sample the total aerosol in the 5 - 100 μm range, without size fractionation.

A preliminary design for such a sampler has been formulated in consultation with scientists at the Institute of Occupational Medicine, Edinburgh (IOM). The design has three main features. Firstly, an upward facing inlet is used to avoid dependence on wind direction. Secondly, a cylindrical wind shield surrounds the inlet. This is intended to create 'calm air' conditions within the shield so as to limit the effect of wind speed on the sampler's aspiration efficiency. Thirdly, a 'rain cap' is placed over the wind shield.

Porous foams were selected as the particle collection medium because:

(i) their low impedance to air flow enables a high volume sampling rate to be achieved using air moving equipment powered from a standard single-phase electrical outlet. This was confirmed by measurements of pressure drop characteristics for a range of foams;

(ii) they have potential for size fractionation.

Although the collection efficiency of porous foams falls off at small particle sizes (typically below 5 μm), this is not a serious drawback because such particles are already effectively sampled. Radiochemical analysis of the foams would require them to be ashed. Tests were therefore conducted to confirm that the material could be ashed at a temperature below 450°C, necessary to avoid polymerisation of plutonium.

After the preliminary design work was carried out, a contract was placed with IOM to produce a detailed design for the sampler on the basis of which a prototype could be built, and to consider in detail the use of porous foams as the particle collection medium. In this design study, considerations of particle losses on the outside of the sampler and of the sampling efficiency of an upward facing inlet in calm air conditions inside the windshield were used to determine optimum values for the dimensions of the sampler. The height and

diameter of the wind shield (1.7m) were chosen to limit losses due to impaction on the external surface. The requirement for representative sampling for particle sizes up to 100 μm sets a lower limit on the diameter of the inlet itself, although the diameter actually specified (0.3m) was significantly greater than this lower limit. Porous foams are characterised by specifying the 'number of pores per inch' (ppi), which is correlated to the fibre diameter of the foam. It was found that a 50mm thick 30 ppi foam would collect virtually all particles with diameters > 5 μm at an inlet velocity of 2.4 ms^{-1}. For practical reasons, this was the highest face velocity (i.e. the velocity at the surface of the foam) at which the foams could be tested, and the design inlet velocity was therefore limited to 2.4 ms^{-1}, since total particle collection could not be guaranteed at higher inlet velocities. This inlet velocity corresponds to a sampling rate of 10 m^3 min^{-1}.

An approximately 1/2 size version of the large particle sampler, the maximum size suitable for testing in the wind tunnel at IOM, has been constructed jointly by NRPB and IOM, and the IOM wind tunnel facility was made available to NRPB staff for a limited series of tests. Because of the limited time available, measurements were concentrated on conditions representing the severest test of the instrument that could be arranged. This was achieved by using the highest wind speed available, 9 ms^{-1}, and the largest particle size available. Graded fused alumina dust was used with a d_{ae} of 75 μm. A sampling efficiency of approximately 10% was measured, compared with a theoretically predicted value of at least 70%. Although sampling efficiency is expected to increase at lower wind speeds and particle sizes, a value of 10% under the above conditions is not considered acceptable. To understand the reasons for the low efficiency, investigations of the airflow over the sampler were carried out and these were used as the basis for a semi-quantitative description of particle transport into the sampler. The low measured efficiency appears to be caused by the rapid divergence of the airflow upwards from the horizontal direction as it encounters the wind shield. Particles thus have a net vertical velocity away from the inlet which must be overcome before they can be sampled. This factor was assumed to have a negligible effect on sampling efficiency in the original design study, but was not quantified. A new series of wind tunnel tests is therefore being planned using various modified windshields designed to present a smaller or more streamlined cross-section to the wind. Sampling efficiency should also improve if higher inlet velocities are used. This will require the determination of the particle collection efficiency of porous foams at these higher face velocities.

Some preliminary work has also been carried out at NRPB to investigate the possible use of porous foams for the size classification of large particles. This would require a multi-layer foam filter with a relatively coarse filter in the uppermost layer collecting most of the larger particles while allowing smaller particles to penetrate. These particles would then be collected by progressively finer foams in the lower layers. Theoretical calculations indicate that, while the cut-off in the collection efficiency vs. particle size curve is not sharp, a suitable choice of foam thicknesses and pore sizes in a multi-layer foam filter would enable particles in the 5 - 100 μm range to be classified into, perhaps, four size ranges. Equipment has been set up to measure the collection efficiency as a function of particle

size for various grades of foams. The dusts are dispersed into an air flow using the Small-Scale Powder Disperser (Thermo Systems Inc.), and the collection efficiency of a foam sample is then determined from the relative weights of powder collected on the foam and on a glass fibre backing filter which collects the powder penetrating the foam filter. Measurements have been carried out on 15mm thick 20 ppi foams with particle sizes in the 6 - 60 μm size range, and preliminary results show a fall off in efficiency with decreasing particle size commencing at about 12 μm. The uppermost foam layer in a multi-layer filter would require 50% penetration at about 50 μm, and so coarser foams are clearly required. Further measurements are planned on 10 ppi foams, and the availability of coarser foams will be ascertained.

(i) Further development and testing of the large particle air sampler. Determination of particle collection characteristics of porous foams at high face velocities, and investigation of their use for particle size classification will continue.

(ii) If adequate sampling efficiency is achieved, a field instrument will be constructed and measurements commenced at a suitable site. Otherwise, effort will be redirected to the development of a rotary impactor.

(iii) Determination of the sampling efficiency of the existing sampler.

Dr J Vincent and Dr D Mark, Institute of Occupational Medicine, Roxburgh Place, Edinburgh, UK.
Contact is also being maintained with Drs Hewitt, Kelly and Harrison at the Universities of Essex and Lancaster with a view to future collaboration, as outlined in the last Progress Report.

None

Title of the project no.: 2

Study of methods for remedying and preventing high radon levels in dwellings.

Head(s) of project:

J C H Miles

Scientific staff:

K D Cliff, J C Strong, R A Algar, P R Lomas

I. Objectives of the project:

To test potential methods for reducing the concentration of radon and its decay products in indoor air and to prevent the occurrence of high radon concentrations in dwellings.

II. Objectives for the reporting period:

To study radon ingress and the effectiveness of remedies developed with the Building Research Establishment for a representative range of conditions found in occupied dwellings in the UK. To study the magnitude and mechanisms of radon ingress through various floors by means of test structures erected on phosphate sand. To study the effect of pressure differentials and environmental parameters on radon ingress for floors constructed conventionally and for floors designed to prevent radon entry.

III. Progress achieved:

The test site has been excavated and filled with phosphate sand: see the previous reports. Two structures have been erected on the phosphate sand, one having a monolithic concrete slab floor construction with the structure-supporting walls built off this slab, the other with a ring-beam foundation to carry the supporting walls. The structure with the slab floor is used as a reference, and no alterations to this are planned, whereas the structure with the ring-beam foundation (the test structure) is being fitted sequentially with a variety of floor types. Both structures have been equipped with instruments to record radon concentration and the values of various environmental parameters including the differential pressures developed across components of the structures. A weather station has been erected at the site to record wind speed and direction, ambient temperature, and absolute atmospheric pressure. All parameter values are being automatically recorded at fixed time intervals. A variable-speed ventilation fan has been fitted to each structure permitting control of reduced or excess pressures relative to atmospheric to be maintained in the structures. Adjustable leakage areas in the fabric of the structures have been provided, which in conjunction with the variable-speed fan, allows ventilation rate and pressure differentials to be varied independently.

The radon concentration in the interstitial pores of the phosphate sand at depth exceeds 10^5 Bq m^{-3}. To determine the maximum radon concentration attainable in the test structure, initial measurements were carried out while the sand was exposed fully. Under these conditions and with ventilation rate $<0.2\ h^{-1}$, the steady-state radon concentration was about $6\ 10^4$ Bq m^{-3}; here, diffusion was the dominant mode of radon entry. That a substantial pressure-induced flow of radon into the test structure from the underlying sand could be created was demonstrated by maintaining conditions such that the ventilation rate was approximately $0.2\ h^{-1}$ and the pressure within the structure was about 5 Pa below atmosphere. Under these conditions, the rate of radon entry into the structure was found to be 4.4 kBq m^{-3} h^{-1} of which 1.1 kBq m^{-3} h^{-1} was attributable to diffusion. There is, therefore, an adequate supply of radon to the test structure for the efficacy of different floor constructions as barriers to radon ingress to be assessed.

At the end of this reporting period, a suspended timber floor of traditional construction was installed in the test structure and air bricks fitted in two opposing sides to vent the underfloor space. Initial results for natural underfloor ventilation point to the possibility that an order of magnitude reduction can be achieved under favourable meteorological conditions.

During this reporting period, work on remedial and preventive measures with the Building Research Establishment (BRE) culminated in the publication of The Householders' Guide to Radon offering advice on the risks from radon and on methods of preventing radon entry, and also of Interim Guidance on Constructions in Radon-Prone Areas which specified the radon preventive measures required in new dwellings. New dwellings are now being erected in southwest England with floor constructions complying with the interim guidance. Consideration is being given to the possibility of determining how effective the new forms of floor constructions are in preventing radon entry and in particular how the standard of workmanship affects the outcome.

Physical remedial measures are being undertaken by BRE in a number of homes where the radon concentration exceeds the Action Level of 400 Bq m^{-3} as confirmed by extensive monitoring. The remedial measures have been designed by BRE in consultation with NRPB, which also carries out post-remedial monitoring of radon levels to determine the effect of the measures. No results of the monitoring are available at the time of this report. Investigations in a number of houses have shown, however, that high radon levels could be reduced somewhat by minor modifications. Open fires, for example, are often on the ground floor of homes having suspended timber floors with little provision being made for a supply of fresh air at that level, thus creating a large reduction in pressure and a consequent influx of radon-laden air from the underfloor space. Provision of modest ventilation at the ground floor level would alleviate this condition. Other such approaches to the problem are also being considered.

IV. Objectives for the next reporting period:

The studies with the test structure built on phosphate sand will continue. The values of parameters affecting radon entry into this structure with the suspended timber floor will be determined. A two-compartment model of air interchange between the underfloor space and that above the floor will be tested in cooperation with BRE. Methods of reducing radon entry through this timber floor will be investigated. The timber floor will then be replaced by suspended concrete of standard design, and effective treatments for this type of floor to improve its properties as a barrier to the ingress of radon will be developed. Measures to reduce the radon concentration in dwellings of diverse types will be installed under the supervision of BRE, and the effectiveness of these measures will be determined. Realistic assessment of the costs of different approaches to the reductions will be made.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

The Building Research Establishment
Garston
Watford
Herts WD2 7JR

Dr C Uglow, Dr S Wozniak

VI. Publications:

The householders' guide to radon. June 1988. Department of the Environment. HSMO, London.

The origin of indoor radon. A D Wrixon, S L Wan and K D Cliff. Presented at the 7th International Conference of IRPA, Sydney, April 1988.

UK standards for exposure to radon daughters in dwellings. G A M Webb and M C O'Riordan. ibid.

Building Regulations: Precautions against radon in new housing. June 1988. Department of the Environment, London.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

CEN-IPSN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-119-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. N. Parmentier
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F-92265 Fontenay-aux-Roses

Telephone number: (1) 46.54.83.35

Title of the research contract:

Assessment of population doses from accidental releases of radioactivity and socio-economic cost of evacuation.

List of projects:

1. Establishment of a method to evaluate the socio-economic cost of an evacuation of the population following a nuclear accident.
2. Evaluation of absorbed doses due to external exposure to photons emitted by a radioactive cloud following an accident and assessment of the protection of dwellings.
3. Evaluation of population dose preceding or following accidents involving releases of gases and radioactive aerosols into the atmosphere.

TITRE DU PROJET n°1 : CONTRAT B16-0119-F(CD)

Establishment of a method to evaluate the socio-economic cost of an evacuation of the population following a nuclear accident.

CHEF(S) DE PROJET : J.BRENOT, N.PARMENTIER

EQUIPE SCIENTIFIQUE : J.BRENOT, F.RANCILLAC

I. OBJECTIFS DU PROJET

Le choix des mesures de protection pour les populations en cas d'accident radiologique tient compte bien évidemment des risques sanitaires potentiels, mais aussi des problèmes de mise en oeuvre et des conséquences économiques et sociales qu'impliquent ces mesures. Ces deux derniers points deviennent des éléments prépondérants lors de la prise de décision quand les risques sanitaires potentiels se situent dans une plage intermédiaire entre le seuil où l'évacuation n'est pas envisagée et le seuil où elle devient impérative. L'objectif du projet est, dans l'optique d'une évacuation, de mettre au point une méthode d'aide à la décision qui s'appuie sur une quantification des conséquences économiques et sociales.

II. OBJECTIFS DE LA PERIODE :Jan.- Dec. 1988

La fin du contrat était prévue pour Juin 1988. Il a été demandé en Juin une prolongation de six mois qui a été accordée. La présente année a été consacrée à la synthèse des points de vue économiques et sociaux.

Le rapport final "Conséquences socio-économiques d'une évacuation de population" présente une méthodologie structurée pour la prise en compte des aspects économiques ; il rassemble, au delà des effets sanitaires, des données sur les éléments essentiels du coût social d'une évacuation, à savoir les opinions et intentions des individus dans le cas d'une possible évacuation ainsi que leurs comportements lors d'évacuations passées ; il aborde, du côté des décideurs, les problèmes d'information et de communication que pose toute évacuation et qui, plus ou moins bien résolus, renforcent ou diminuent la crédibilité des actions et des autorités.

Le rapport traite d'abord d'évacuations historiques en soulignant les problèmes qui se sont posés. Les plans d'urgence sont ensuite étudiés pour voir quel est le poids de l'Economique et du Social lors de la gestion de la crise. Les méthodes économiques sont développées et appliquées dans le cas de zones où les activités concernent prioritairement l'industrie et la prestation de services, donc de zones urbaines ou semi-urbaines plus ou moins industrialisées. Enfin, les aspects sociaux sont présentés. Et c'est l'approche multi-critères qui est préconisée pour la prise en compte des dimensions économiques et sociales lors de décisions.

VI. REFERENCES

* Assouline M., Bastien M.C., Brenot J., Dumas M., Parmentier N. Economic consequences of evacuation in industrialised urban areas. Radiation Protection Dosimetry,1987, Vol.21, N°1/3, pp.165-169.
* Brenot J. Conséquences socio-économiques d'une évacuation de population. Rapport IPSN/DPS, Déc.1988.

Title of the project no:

ABSORBED DOSE ASSESSMENT FOR EXTERNAL EXPOSURE TO PHOTONS EMITTED IN RADIOACTIVE CLOUDS AFTER ACCIDENTAL RELEASES DWELLING PROTECTION FACTORS ASSESSMENT

Head(s) of project:

J. LE GRAND

Scientific staff:

J. Le Grand, Y. Roux, N. Parmentier, Nguyen Van Dat, C. Madelmont, P. Boulsset, D. Robeau, G. Kerlau

I. Objectives of the project:

When accidental radioactive releases occur, countermeasures may be decided on :

- assessment of whole body dose for external exposure to radioactive cloud or radionuclide deposited ;
- assessment of protection factors, i.e. dose reducing ratio if sheltering of people in dwelling is planned.

II. Objectives for the reporting period:

- Dose data base for immersion in cloud of radionuclide releases in open air.
- Collaboration with GSF.
- Sampling of representative buildings around all the French nuclear sites and calculation of their shielding factors.
- Shielding factor data base for radionuclide releases on each nuclear sites.
- Comparison of reducing kerma ratio and reducing absorbed dose ratio.

1. Methodology

For each "département" and each photon initial energy a distribution of protection factors has been determined. This distribution allows to calculate several representative average protection factors per household, per building, per dominant housing type which represents more than 50 % of the households in the "département".

In order to determine a realistic and conservative protection factor for a radionuclide in each "département" the classification of these average values are studied according to the photon initial energies.

2. Results

1 - Dose factors data base

Kerma rates at 1 m and .5 m above ground level has been calculated in open air for immersion in a semi-infinite cloud and for exposure to an infinite plane source in urban, rural and aquatic environments. 115 radionuclides are considered.

2 - Protection factors data base

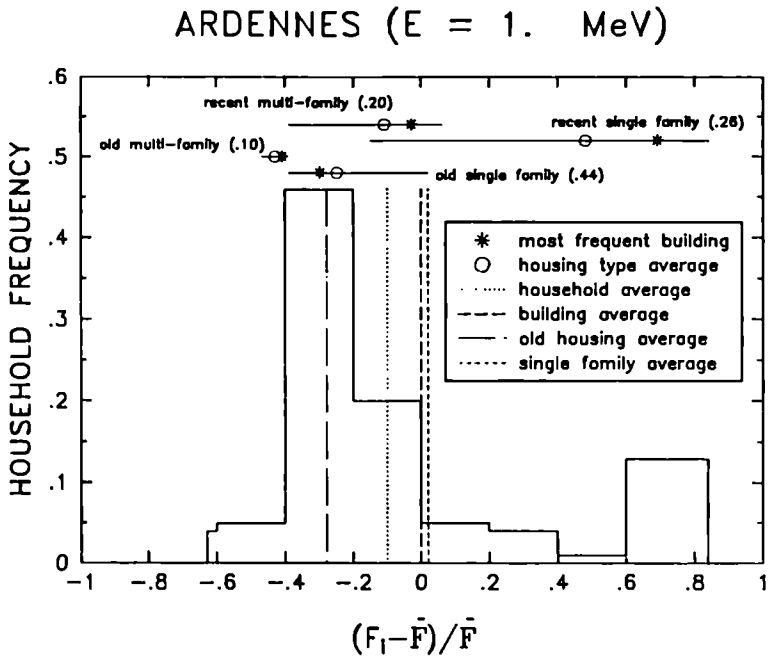
The results of the statistical survey of housing characteristics in 31 "départements" in FRANCE had allowed to define a sample of 647 buildings (about 20 in each "département"). Protection factor in each of them has been calculated for six photon initial energies : .1 - .2 - .5 - 1. - 2. and 5 MeV.

Protection factors per floor and per building are classified in the data base which has been set up in 1988.

The results of the first experimental campaign, made in 1987, had shown that the mean kerma rate, calculated with our computer code BILL, are overestimated in small inhabitable volumes, underestimated in great inhabitable volumes ; but it is best estimated in the most frequent rooms (inhabitable surface about 12 m^2). Then a systematic study on the sample of buildings had shown that the mean kerma rates per floor obtained with the computer code BILL, must be multiplied by a factor which is always included in the range .9 to 1.1.

3 - Protection factors distributions

The figure presents an example where protection factor is considered as a standardized variable to allow comparison with other photon initial energies and other "département". The protection factor distribution in the households of the building sample is the full line curve. On the upper part of the figure are presented the range of the protection factor per building for each housing type (number between brackets is the household frequency for the housing type). The vertical lines are the protection factor averages which are representative of the housing in ARDENNES. For this example, the single family average may be considered as a conservative and realistic protection factor.



4 - Collaboration with G.S.F.

A report on dosimetric measurements made during the two experimental campaigns (September and October 1987) completed with dosimetric and spectrometric calculations made with our computer codes has been sent to Gesellschaft für Strahlen-und-Umweltforschung (G.S.F.) on July 1, 1988. This report must be completed by comparison with G.S.F. results, particularly with spectrometric measurements made in October 1987 when we shall receive them.

3. Discussion

1 -The method used to calculate the kerma rate average in a household has been adopted to reduce the calculation time as much as possible. This method, based on a geometrical data describing the inhabitable volumes of a household, has been verified by the experiments (September 1987). Two main conclusions can be expressed :

- the errors due to the calculation method of the kerma rate average in a household are generally, less than 10 %,

- the method cannot be used to study the kerma rate distribution inside a household.

2 - For external exposure, the effective dose equivalent is determined from the ratio of organ absorbed dose to kerma. This ratio has been calculated by POSTON and SNYDER and later, by ECKERMAN and altri which have considered both, a semi-infinite cloud without soil. Our calculations in the energy range of .01 MeV to 5 MeV agree, for a semi-infinite cloud without soil, with POSTON and SNYDER results, and for a man standing on concrete soil with ECKERMAN results.

IV. Objectives for the next reporting period:

- Study of the protection factor distributions in French "départements".
- Influence of housing characteristics on protection factor.
- Conservative and realistic protection factors against cloud exposure for 115 radionuclides.
- Collaboration with G.S.F. will be continued.
- Action on deposit on ground and building surfaces will be continued. Especially research on historical evolution of spatial organisation in town planning will begin to define representative models.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]

- J.P. PATAU, **Faculté de Pharmacie** - Service de Biophysique, Université Paul Sabatier - 35 rue des Maraîchers - 31062 TOULOUSE Cedex

- J.C. CROIZE - **C.R.H.** (Centre de Recherche sur l'Habitat, associé au Centre National de la Recherche Scientifique (UA 1248)) - Ecole d'Architecture de PARIS LA DEFENSE - 41 allée Le Corbusier - 92023 NANTERRE Cedex

VI. Publications

- J. LE GRAND, Y. ROUX, G. KERLAU
Expériences de validation des codes de calcul utilisés pour déterminer les facteurs de protection apportés par les habitations.
Rapport CEA-R-5464.

- J. LE GRAND, Y. ROUX
Facteurs de dose pour l'irradiation externe : Immersion dans un nuage et dépôt surfacique de radionucléides,
Rapport DPS-88/07-SEAPS.

Title of the project no.:

EVUALATION OF POPULATION DOSE PRECEDING OR FOLLOWING ACCIDENTS INVOLVING RELEASES OF GASES AND RADIOACTIVES AEROSOLS INTO THE ATMOSPHERE.

Head(s) of project:

Dr. N. PARMENTIER
CEN-FAR IPSN BP N°6
F-92265 FONTENAY AUX ROSES

Scientific staff:

I. Objectives of the project:

L'objectif de ce projet est le développement d'un code de calcul de transfert atmosphérique permettant de prendre en compte des situations complexes.Cette modélisation est basée sur la résolution tri-dimensionnelle de l' équation de diffusion-convection par une méthode de suivi de particules.Les incertitudes sur les différents paramètres intervenant dans la modélisation doivent pouvoir être pris en compte dans la méthode présentée.Cette prise en compte de l'incertitude se traduit par la transformation des paramètres de la modèlisation en variables aléatoires dont la fluctuation est régie par une distribution de probabilité.

II. Objectives for the reporting period:

Durant la période couverte par le présent rapport (juillet 1988 à juillet 1989) une méthode d'analyse d'incertitude a été introduite dans le code de calculs des transferts atmosphériques.Au lieu de prendre une valeur déterminée, chacun des paramètres se voit associer une distribution de probabilité.Lors du calcul, à chaque fois qu'un paramètre est nécessaire, tels par exemple les vitesse et direction du vent, les vitesses de dépôt sec, les taux de lessivage..., la valeur du paramètre est tirée aléatoirement suivant une distribution de probabilité déterminée à l'avance.

III. Progress achieved:

METHODOLOGIE:

Le travail effectué durant cette période est plus précisement décrit dans le rapport cité en référence.Le code de calcul du transfert atmosphérique écrit lors de la dernière période a été modifié de façon à ce que la valeur déterminée des paramètres soit remplacée par une recherche stochastique et heuristique dont les fluctuations répondent à une loi de probabilité.
Afin d'éviter que les "hasards" des tirages aléatoires fassent dévier telle ou telle distribution de probabilité, un échantillonage par une méthode d'hypercube latin a été choisi.Cette méthode permet d'avoir la certitude que les valeurs numériques tirées au hasard décrivent la totalité de cette distribution de probabilité qui leur est associée.On peut associer à chaque paramètre un type de distribution de probabilité parmi les suivantes: distribution uniforme, distribution normale, distribution log-normale, distribution triangulaire et distribution β-incomplète.
Les calculs de radioactivité atmosphérique et de dépôt au sol effectués avec cette méthode permettent d'intégrer au calcul les fluctuations du vent tant en direction qu'en vitesse, les incertitudes spatiales sur les vitesses de dépôt, les taux de lessivage, et les coefficients de dispersions verticales et transversales.

RESULTATS 1988:

Pour calculer la radioactivité atmosphérique et les dépôts, on a utilisé l'équation de la diffusion convection en milieu homogène résolue par une méthode stochastique.Le terme source a été choisi ponctuel dans l'espace et dans le temps.
Chacun des paramètres du modèle:
-direction et vitesse du vent
-vitesse de dépôt au sol
-coefficients de diffusion latéral et axial,
ont été associés à une distribution de probabilité uniforme puis à une distribution de probabilité normale.

Les calculs de dispersion atmosphérique ont été effectués tout d'abord à paramètres constants, puis on a permis à tous ces paramètres de fluctuer dans les limites définies par les distributions de probabilités.Une nouvelles série d'expérimentations numériques a été effectuée, en permettant à tous les paramètres de fluctuer sauf un.Cette technique a permis de mettre en évidence les différences numériques observées entre un calcul à paramètres fixes et un calcul à paramètres fluctuants.La seconde série de tests a permis de quantifier l'effet de fixité d'un paramétre lorsque tous les autres varient.

Les effets sur les valeurs de dépôts et de radioactivité atmosphérique maximum ont été observés, ainsi que les écarts de temps de transfert entre le point source et le point d'activité maximum.

En faisant varier les caractéristiques des distributions de probabilité, la variabilité des paramètres a été étudiée en permettant à tous les paramètres de varier :
-de 10,100 puis 500% pour les vitesses de dépôt,
-de 10 puis 100% pour les coefficients de dispersion,
-de 10 puis 50% pour les vitesses de vent,
-de 10,20 puis 30°pour les directions de vent,
de chaque côté de leur valeur médiane.

DISCUSSIONS:

Les analyses d'incertitudes qui ont été éffectuées ont permis d'observer plusieurs effets qui vont dans le sens d'une diminution de la radioactivité atmosphérique et des dépôts au sol calculés, agrémentée d'une augmentation des surfaces concernées.Ces remarques sont en concordance avec les mesures expérimentales qui montrent des hétérogénéités importantes même lorsque les conditions apparaissent homogènes.Il apparait donc que ce type de méthode qui tend à substituer une distribution de probabilité à l'ignorance du comportement spatiale d'un paramètre apporte une amélioration de l'estimation de la radioactivité de l'air et des dépôts.Cependant il est évident que ces observations doivent être confortées par de nouvelles expérimentations numériques, si possible couplées avec des expériences de rejets concertés dans l'atmosphère.

IV. Objectives for the next reporting period:

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

(2)-Rapport DPS 88/02 SEAPS Analyse de sensibilité: Formulation stochastique.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-130-D

Isotopenlaboratorium f. biologische
und medizinische Forschung der
Georg-August-Universität
Burckhardtweg 2
D-3400 Göttingen

Head(s) of research team(s) [name(s) and address(es)]:

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Isotopenlaboratorium
Georg-August-Universität
Burckhardtweg 2
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Telephone number: (551) 39.81.02

Title of the research contract:

The aerosol size distributions and the unattached fraction of the radon daughters for estimation of the radiation exposure risks in houses.

List of projects:

1. The aerosol size distributions and the unattached fraction of the radon daughters for estimation of the radiation exposure risks in houses.

Title of the project no.:

The aerosol size distribution and the unattached fraction of radon daughters for estimation of the radiation exposure

Head(s) of project:

Dr. J. Porstendörfer

Scientific staff:

Dr. A. Reineking, Dipl.-Phys. K.H. Becker,
Dipl.-Phys. G. Butterweck

I. Objectives of the project:

In all dosimetric models the aerosol particle size and the unattached fractions of the radon daughters are important parameters for the estimation of the radiation exposure to the human body. By means of different measuring techniques (high-volume impactors, low pressure impactors and screen diffusion batteries) the activity size dsitributions, the size distribution of the non-radioactive aerosols and the influence of the natural concentration and aerosol sources have to be measured in the diameter size range between 0.5 and 10000 nm in houses and in the open air. Model calulations have to be performed to determine specific parameters (plateout rates, attachment rates, etc.) and the radiation exposure.

II. Objectives for the reporting period:

The calibration of the low pressure impactor with monodisperse aerosol particles by varying the pressure of the last stages will be finished. The measurements of the activity size distributions of the aerosol attached shortlived radon daughters in the outdoor atmosphere will be continued with the low pressure impactor, and it is also planned to measure the outdoor mass size distribution. With the screen diffusion battery technique the fraction of the unattached activities will be determined in the outdoor environment.
Intercomparison measurements with other groups will be repeated in a house with higher unattached fractions.

III. Progress achieved:

1. Methodology

A low pressure cascade impactor (type Berner) was calibrated with monodisperse, liquid radioactive labelled aerosol particles for two different pressures (180 and 245 mbar) behind the last impactor stage. The efficiencies of the different stages were measured for aerosol particles with diameters between (50 - 300) nm. In the ultrafine size range of atoms or ions (≈ 1 nm) the response function of the impactor was measured with unattached atoms of the thoron decay product ^{212}Pb.

In the surroundings of Göttingen outdoor activity size distributions of the shortlived radon decay products ^{214}Pb and ^{214}Bi were measured with the low pressure cascade impactor. First measurements of the unattached fractions of the radon daughters ^{218}Po and ^{214}Pb were performed in the outdoor atmosphere with the screen diffusion battery technique in connection with the α-spectroscopy.

Different experimental methodologies for the determination of the dynamics of radon dauhgters in realistic indoor environment were compared by means of joint measurements performed in a house with elevated radon gas concentrations in North Bavaria. Groups of the Gent State University (Belgium), the National Radiation Protection Board (Chilton, England) and the Zentrales Isotopenlabor of the University of Göttingen (FRG) have been participating at this European Intercomparison of radon daughters measuring equipment.
The intercomparison measurements were performed in a house with higher radon gas concentrations up to 2000 Bqm^{-3} under various aerosol conditions. At the first day the behaviour of the radon daughters was measured in a closed room with an aged aerosol. At the following days the influence of aerosol particles of different sizes produced by a burning candle, by gas heating and smoulding cigarettes was studied. In addition to the measurements of the radon gas and radon decay product concentrations in the air, the activity size distribution was measured by two different screen diffusion battery techniques and by a low pressure cascade impactor. Meanwhile the inactive distribution was measured by a third screen diffusion battery technique and by a differential mobility analyser method.

Results

The low pressure impactor consists of eight stages and operates at a volumetric flowrate of 1.8 m^3h^{-1}. The efficiency curve of the last stage was changed by varying the pressure at the exhaust of the impactor, i.e. changing the mean free pathway of the aerosol particles. The influence on the efficiency of the other stages can be neglected. For liquid particles a 50 % - cut-off diameter of 82 nm was measured at 180 mbar and one of 112 nm at 245 mbar.
Calibration measurements carried out with unattached ^{212}Pb show that about 75 % of the unattached activity is lost in the entrance or on the walls of the first three impactor stages.

In the outdoor atmosphere the activity size distribution of the shortlived radon decay products was measured about 1 m above the ground. Some mass size distributions were determined using extended sampling periods of about 3 days.
The activity median aerodynamic diameter (AMAD) of the accumulation mode (69-100 % of the total activity) varied between 173 and 645 nm with a mean value of 369 nm. Due to outdoor aerosol sources (resuspension and combustion processes) sometimes activities in the nuclei mode (< 100 nm: 0-15 %) and coarse mode size range (> 2000 nm: 0-25 %) were measured. The mass median aerodynamic diameter (MMAD) of the accumulation mode ranged between 400-500 nm (≈ 70 % of the total mass) and the remaining 30 % of the mass was found in the coarse mode size range with median diameters of about 5000 nm.

Preliminary measurements during the daytime show that in the surroundings of Göttingen the unattached fraction f_p of the radon decay products ranges up to 0.2 with a mean value of 0.02 at a mean aerosol particle concentration of $34*10^3$ cm^{-3} (range: (8-100) $*10^3$ cm^{-3}). During this measurements the equilibrium factor ranges between 0.2-1.2 with a mean value of 0.7.

The data evaluation of the European Intercomparison measurements has not yet finished.

3. Discussion

The measured outdoor activity size distribution of the aerosol attached shortlived radon daughters agree with our former high-volume impactor studies. The mean AMAD of the main activity mode (accumulation mode) of the outdoor size distribution (AMAD=369 nm) is significantly shifted to greater diameters compared with the indoor results (aged aerosol: AMAD=188 nm) due to different plateout and coagulation rates. These results also agree with measurements by Sinclair et al. where 150 nm was determined indoors and about 500 nm outdoors.

Due to higher aerosol particle concentrations and higher attachment rates the measured mean unattached fraction f_p of 0.02 in the open atmosphere is essentially smaller than those measured in closed rooms without additional aerosol sources (f_p=0.10).

These results of the median diameters of the aerosol attached activities indoors and outdoors and of the amount of the unattached activities f_p show that most dosimetric models in the estimation of the radiation exposure of the human public based on incorrect data. In these model calculations mean unattached fractions f_p of 0.03 are used, and it is assumed that the particle size of the attached activities in the indoor atmosphere is greater than in the open atmosphere.

IV. Objectives for the next reporting period:

The measurements of the properties of the shortlived radon decay products and of the mass will be continued in the outdoor atmosphere.
A thoron gas monitor must be calibrated, and it is planned to perform first measurements in rooms with higher thoron gas levels.
Properties of the thoron decay product ^{212}Pb (ThB) will be measured by the low pressure cascade impactor and by the screen diffusion battery technique indoor as well as outdoor.
The data evaluation of the European intercomparison measurements will be finished. The results will be published in the next reporting period as a European report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1) H. Vanmarcke and R. Van Dingenen
Gent State University, Nuclear Physics Laboratory
Proeftuinstraat 86
B-9000 Gent, Belgium

2) J. Strong
National Radiation Protection Board
Chilton, Didcot
Oxon OX11 ORQ, England

VI. Publications:

Reineking, A. and Porstendörfer, J., "Activity size distributions of the shortlived radon decay products and their influence on the deposition probability in the human lung", European Aerosol Conference, 30 August - 2 September 1988, Lund, Sweden
J. Aerosol Sci. 19, 1331-1337, 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-107-DK

Risø National Laboratory
Health Physics Department
DK-4000 Roskilde

Head(s) of research team(s) [name(s) and address(es)]:

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Health Physics Dept.
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DK-4000 Roskilde

Telephone number: (2) 37.12.12

Title of the research contract:

Behaviour of accidentally released radionuclides in urban areas.

List of projects:

1. Behaviour of accidentally released radionuclides in urban areas.

Title of the project no.:

Experimental and modelling approach to assess indoor doses in urban agglomerations, and evaluation of the decontamination through run-off of deposited material.

Head(s) of project:

Jørn Roed

Scientific staff:

H.L. Gjørup
P. Hedemann Jensen
J. Roed
F. Heikel Vinther

I. Objectives of the project:

To establish a more comprehensive methodological approach for assessing the consequences of accidental releases of radioactive material in urban areas. This is done by investigating some important related phenomena: the reduction in inhalation dose by staying indoors, deposition of contaminant on indoor and outdoor surfaces, run-off effect, shielding factor for houses etc.

II. Objectives for the reporting period:

Finding dry deposition velocities on pervious urban surfaces, especially trees and lawns, and comparing them with those in rural and forested areas. Finding and comparing indoor deposition parameters in furnished and unfurnished rooms. Following the weathering processes on roof materials in order to quantify them.

III. Progress achieved:

Dry deposition on rough surfaces such as trees and lawns has been studied. It is found that the deposited material is distributed fairly uniformly on trees as seen in Table 1, and that the deposition velocity is nearly proportional to the mass of the bulk material (small branches, twigs, and needles) per unit projected area. Therefore, it is surgested that a bulk deposition constant be used when modelling deposition on trees. The bulk deposition constant, B_d, is defined as the amount of deposited material per unit bulk mass divided by the time-integrated air concentration. The bulk deposition constants for different trees are shown in Table 2.

Table 1. Deposited material on small branches, twigs, and needles of common spruce: Bq/kg of small branches, twigs, and needles.

Height	Isotope							
cm	^{95}Nb	^{103}Ru	^{106}Ru	^{131}I	^{134}Cs	^{137}Cs	^{141}Cs	^{144}Ce
0-75	22.8	5.5	0.8	226.7	1.1	3.1	9.1	6.0
75-135	57.7	11.2	3.7	181.0	1.2	3.0	24.0	18.2
135-215	32.6	6.1	1.8	162.4	1.2	2.5	30.1	14.4
215-315	16.5	10.5	2.3	173.4	1.9	4.2	13.3	8.5
315-405	39.7	7.0	1.6	-	1.5	3.7	20.5	14.4
405-472	34.8	11.5	3.4	121.7	1.5	3.3	26.0	18.0
472-545	27.3	10.5	3.4	229.6	2.1	4.6	22.5	16.8
545-654	39.4	12.2	4.9	72.8	1.8	4.6	20.2	10.4

Table 2. Bulk deposition constant, B_d: 10^{-4} m^3 s^{-1} kg^{-1}

Isotope	Yew trees height 2.5 m	Jupiter berry height 2.0 m	Common spruce height 6.5	Common spruce height 6.1 m
^{137}Cs	2.8	3.2	1.8	2.3
^{134}Cs	2.2	2.7	1.4	1.9
^{131}I	24.5	26.5	19.4	16.6
^{141}Ce	12.2	21.9	13.9	19.4

Table 3 shows a comparison of the deposition velocity, V_d, and the bulk deposition constant, B_d, for grass. It is seen that V_d changes much more rapidly than B_d from sample to sample. The bulk deposition constant therefore often proves to be a more useful parameter than the deposition velocity when modelling deposition on grass surfaces.

Table. 3. Grass.
Deposition velocity, V_d: 10^{-4} ms^{-1},
Bulk deposition constant, B_d: 10^{-4} $m^3s^{-1}kg^{-1}$

Sample No.		^{137}Cs	^{134}Cs	^{131}I
1384	V_d	4.3	4.4	22
	B_d	21	21	110
1387	V_d	1.8	1.5	18
	B_d	10	8.7	100
1388	V_d	8.8	7.2	93
	B_d	10	8.5	110
1391	V_d	6.0	6.6	86
	B_d	7.9	8.7	110
1392	V_d	7.4	9.9	120
	B_d	9.1	12	140

Beryllium-7 particles are used as tracers for investigating the deposition of small particles on surfaces in a furnished and unfurnished house

The experimental set-up provided us with information about λ_d, the rate coefficient of deposition (the fraction of the aerosols in the building deposited per unit time). Knowing λ_d, it is possible to find an average local deposition velocity, U_d, defined as $U_d = \lambda_d \cdot V/A$ where V is the total volume of the room considered and A the internal surface of walls, roof and floor in the room.

As shown in Table 4, the deposition velocity in the unfurnished room is about 3 times less than in the furnished room. The deposition velocity for the partly furnished house is in-between. The higher deposition velocity for ^{137}Cs-marked particles as compared to ^{7}Be-marked particles is due to the larger particle size of the former as they stems from the resuspended material.

Table 4.

	Isotope	Deposition velocity, U_d:10^{-4} ms^{-1}	Rate coefficient of deposition, B_d:h^{-1}
partly furnished house	^{7}Be	0.18±0.05	0.11±0.03
	^{137}Cs	1.5 ±0.3	0.91±0.16
unfurnished room	^{7}Be	0.10±0.06	0.06±0.03
furnished room	^{7}Be	0.33±0.08	0.21±0.05

The weathering and run-off from roofs has been studied, and it is found that the weathering process is very slow when considering cesium retained during the Chernobyl accident.

IV. Objectives for the next reporting period:

Measuring dry deposition velocities on impervious urban surfaces and on the basis of these and earlier measurement, finding a relative distribution of the dry deposited material on the different urban surfaces.

Continue the measurements of run-off and weathering and from the total amount of data available to construct a dynamic model for run-off from and weathering of roof material.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Roed, J. (1987). Dry deposition in rural and in urban areas in Denmark. Radiation Protection Dosimetry, Vol. 21 No. 1/3 pp 33-36 1987.

Roed, J. (1987). Run-off from and weathering of roof material following the Chernobyl accident. Radiation Protection Dosimetry, Vol. 21. No. 1/3 pp 59-63 1987.

Roed, J. and R.J. Cannell (1987). Relationship between indoor and outdoor aerosol concentration following the Chernobyl accident. Radiation Protection Dosimetry, Vol. 21,No. 1/3 pp 107-110 1987.

Roed, J. (1988). Parameters used in consequence calculations for an urban area, NKA/AKTU 245(88)1. Presented at the Joint CEC/OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy 25th-29th January, 1988.

Roed, J. (1988). The distribution of dry-deposited material on trees from the Chernobyl accident. NKA/AKTU-245(88)2. Joint CEC/ OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy 25th-29th January, 1988, Proceedings of the second part of the workshop, p 165-178.

Roed, J. and R.J. Cannell (1988). The deposition of beryllium-7 marked particles on surfaces in unfurnished and furnished rooms, NKA/AKTU-245(88)3. Joint CEC/OECD (NEA) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy, 25-29 January, 1988, Proceedings of the second part of the workshop, p 208-221.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-120-NL

Kernfysisch Versneller Instituut
FOM
Van Vollenhovenlaan 661
NL-3527 JP Utrecht

Head(s) of research team(s) [name(s) and address(es)]:

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Kernfysisch Versneller Instituut
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Telephone number: (50) 63.36.00

Title of the research contract:

Investigation of the mechanisms leading to radon concentrations in dwellings.

List of projects:

1. Investigation of the mechanisms leading to radon concentrations in dwellings.

Title of the project no.:

Investigation of mechanisms leading to radon concentrations in dwelings.

Head(s) of project:

L.W. Put

Scientific staff:

F.J. Aldenkamp
R.J. de Meijer
L.W. Put

I. Objectives of the project:

- Development of instruments for measuring radon exhalation rates and time-averaged radon concentrations;
- Study of the transport of radon in materials and in dwellings;
- To perform measurements of radon exhalation rates and radon concentrations with the aim to get a better understanding of the physical phenomena that lead to radon concentrations indoors, with emphasis on the influence of the crawl spaces.

II. Objectives for the reporting period:

- Continued study of the mechanisms causing the large uncertainties in the determination of the exhalation rate;
- Construction and tests of a new exhalation meter;
- Measurements of exhalation rate in dwellings and from soil;
- Continuation of radon-concentration measurements in "high-radon" houses;
- Exploring the influence of ground water;
- Measurements of radon concentration in soil gas and radiometric analysis of soil samples of "high-radon" houses;
- Improvement of the activated-charcoal cannister technique.

III. Progress achieved:

1.Processes influencing the detection mechanism of a radon exalation meter.

1.a. Methodology and history

In-situ measurements of radon exhalation by surfaces like soil or walls in dwellings may be carried out by placing a hood on the surface and measuring the growth of the radon concentration in the hood by collecting the radon decay products on a foil and measuring their α-decay.

The project started with the investigation of the detection properties with a prototype exhalation meter. It was found that the growth curve showed fluctuations which gave uncertainties of a factor 2 to 3 in the deduced value of the radon exhalation. By the end of 1987 a revised version of the device was designed and constructed to investigate the mechanisms causing the fluctuations under controlled conditions.

The emphasis of the research in 1988 has been the investigation of the mechanisms influencing the collection mechanism in order to define a more definite design of the device and to deduce conditions under which reproducable and reliable exhalation values may be obtained.

The changes in the design of the prototype involved a modification of the electrostatic field, the use of a solid state detector instead of a ZnS scintillator and the possibility to continuously monitor relative humidity and temperature inside the device. Moreover feed-throughs were installed to allow to change the absolute humidity and to sample the radon concentration with a Lucas cell. In addition the device was placed in a temperature controlled environment and the source of radon was changed from a piece of uranium ore to a radium-chloride solution in a flask. With these changes experiments could be carried out under well controlled conditions. All test experiments were carried out after the device was flushed with argon to suppress possible influences of aerosols.

Since the experiments with the prototype showed hardly any dependence of the collection efficiency on the electrostatic field, the first set of measurements was carried out with a collection voltage $V_{coll} = 100$ V. The temperature was changed from 5 to 35 °C in steps of 5 °C, relative humidity was changed in steps between about 0.2 and 0.9. At each temperature and humidity step, the system was alloved to stabelize (≈4h). After stabelizing, an α-spectrum was recorded from which the ^{218}Po and ^{214}Bi activities on the foil were determined. After recording the spectrum an air sample was taken from the device with an evacuated Lucas cell to measure the radon concentration. Measurements were carried out in the

range between 1 and 10^4 kBq·m^{-3}.

To check whether the new device was still insensitive to the collection voltage, measurements were made at approximately constant radon concentration, temperature and relative humidity. The collection voltage was changed in steps between 100 and 1200 V (the maximum value due to limitations in the electronics). Since it was found that with the new device the collection efficiency is rather sensitive to the collection voltage a set of comparison experiments was carried out at V_{col}= 100 and 1200 V, at constant temperature (20 °C), two values of the relative humidity (0.25 and 0.75), and a number of radon concentrations in the range of 0.5 to 10^3 kBq·m^{-3}.

1.b. Results

Analysis of the data indicated that the dependence on temperature and relative humidity could be reduced to a dependence on the absolute humidity. Fig. 1 shows a three-dimensional representation of the collection efficiency at V_{col}= 100 V for ^{218}Po (ε_1) as function of radon concentration and absolute humidity. One sees that the efficiency is strongly dependent on both variables, especially for low radon concentrations. A similar result is obtained for ^{214}Bi (ε_3); the ratio $\varepsilon_3/\varepsilon_1$, however, turns out to vary between 2.5 and 1.5 depending on concentration and humidity, indicating that the dependencies for ε_1 and ε_3 are significantly different.

Fig. 2 shows that the collection efficiency for ^{218}Po as function of the radon concentration at V_{col}= 100 and 1200 V, at constant temperature and at low and high relative humidity. The measurements indicate that at V_{col}= 1200 V the efficiency is practically constant at concentrations smaller than 10^3 kBq·m^{-3}. Moreover the sensitivity on humidity is much smaller than at V_{col}= 100 V.

1.c. Interpretation

To interprete the results a model was developed in which processes are incorporated responsible for removal of free charged radon decay products in the device. These processe are: neutralization, attachment and electrostatic collection on the foil. It was assumed that 85% of the radon decay products is ionised in α-decay and that in β-decay no ionisation takes place.

The analysis of the results in terms of this model allows a deduction

of values for the neutralization and attachment rates from the experimentally determined efficiencies ε_1 and ε_3. It turns out that neutralization is, of the two, the dominant process. The neutralization rate is comparable to the rate for electrostatic collection at V_{col}= 100 V ($\approx 1\ s^{-1}$). The deduced values of the neutralization and attachment rates turn out to be independent of V_{col}. Increasing V_{col} reduces the effect of neutralization relative to electrostatic collection and consequently it increases the efficiency. Calculations show that an increase in V_{col} from 100 V to 2.5 kV would reduce the neutralization effects to less than 25%.

In literature conflicting results are reported on the effects of neutralization. These differences seem to be related to the experimental conditions. Especially the presence of trace gases with a low ionisation potential, like NO, NO_2 and some organic vapours, may lead to charge transfer and neutralization of the decay products.

1.d. Parametrisation

To convert the count rate to radon concentration the measured efficiencies have been parametrised. It turns out that the efficiencies may be factorised in a product of two functions depending on the absolute humidity, d_v, and the radon concentration, C, to a certain power:

$$\varepsilon_i(V,d_v,C) = F_i(d_v)\cdot C^a.$$

Here i indicates Po or Bi and a has the values -0.32 and -0.07 for V_{col}= 100 and 1200 V, respectively. For $F(d_v)$ a rather simple expression was found: $F(d_v) = a_1 + a_2\ \{\ln(d_v)\}^{a_3}$.
The values of a_i at V_{col}= 1200 V are within the uncertainties equal to the values at V_{col}= 100 V: a_1 = -0.1 ± 0.3, a_2 = -0.1 ± 0.3, and a_3 = 5± 15. These values reflect the effect of the faster electrostatic collection at higher collection voltages. At a constant neutralization rate the shorter residence time for the ions reduces the neutralization probability.

2. Exhalation measurements

By the end of 1988 exhalation measurements were started on surfaces: a gypsum block with a relatively high radium content, a concrete floor and outdoor soil. All measurements have been carried out at V_{col}=1200 V. In all cases the device was flushed with dry nitrogen gas to remove aerosols and trace gases, and temperature, relative humidity and count rates were continuously recorded in a datalogger.

Figures 3 and 4 show measured growth curves for the gypsum block

placed flat on a table and the concrete floor. From the figures it is clear that no fluctuations are present anymore. One further notices that the curve for gypsum flattens off much faster than the curve for the concrete floor. This effect is related to the larger diffusion length in the gypsum block causing escape of radon from the device into the surrounding air via the sample. Both curves have been fitted with a function $f(t) = c + E \cdot A \cdot \lambda^{-1}(1-e^{-\lambda t})$. In this expression E is the exhalation rate, A the area covered and c a constant representing the count rate at t=0. The quantity λ is given by $\lambda = \lambda_\ell + \lambda_{Rn}$, where λ_ℓ and λ_{Rn} are the time constants for leakage and radon decay, respectively.

Table 1 presents the values deduced with the procedure described in section 1. From the table one notices that the leakage constants decrease almost an order of magnitude between block and floor and between floor and soil. The first comparison reflects the difference in diffussion length for radon in the two materials, the second step is most likely due to the much better sealing in the latter measurement.

Table 1. Exhalation rate, E, and the leakage parameter, λ_ℓ, for three samples. The values have been deduced from a fit to the growth curve.

sample	E ($Bq \cdot m^{-2} \cdot h^{-1}$)	λ_ℓ (h^{-1})
gypsum block	22 ± 3	0.61 ± 0.03
concrete floor	7 ± 1	0.045 ± 0.001
outside soil (sand)	5 ± 1	0.0036 ± 0.0005

All results are preliminary and have not yet been corrected for variations in humidity. Especially the results for the soil still suffer from experimental difficulties like condensation and/or freezing of water on the foil, causing e.g. sparks between the foil and the detector housing. Also sometimes fluctuations in the growth curve occur which are larger than expected from changes in temperature and humidity of the air in the device. Possible causes are pressure differences between the device and the outside air, changes in radon exhalation due to varying groundwater levels (see section 5), changes in the humidity of the soil, and/or changes in trace gas concentrations. These effects will require further attention.

3. Activated charcoal canisters

Results presented by Ronca Battista at the Lisboa meeting last year confirm our experience that the collection efficiency of charcoal canisters is strongly humidity and temperature dependent. Promising results reported by others at this conference with foils which stop water vapour but transmit radon have so far not resulted in an improvement of the devices water vapour was indeed prohibited to enter the dosemeter, but the diffusion barrier for radon turned out to be too high for use at radon levels usual in Dutch dwellings.

4 "High-radon" dwellings

The results of the time averaged radon concentration measurements have been analysed in 1988. The project consisted of four dwellings near Arnhem, two sets of dwellings in Maastricht, and five similar houses in Born where various measures were taken to reduce moisture problems. In all dwellings passive radon dosemeters were placed for 3 consecutive periods of 4 months. In addition dosemeters were placed in one of the bedrooms at the first floor, outside the dwellings and as far as accessible in the crawl space. The dwellings were selected on the basis of the observation of relatively high radon concentrations (one in Arnhem and two in Maastricht) in the Dutch radon survey. The other houses were located close to them, they were in Maastricht nearly identical to the houses which participated in the survey.

Comparison of the radon concentration of the four houses in Arnhem indicates that the high radon concentration observed only in the house of the survey is likely due to the specific properties of that dwelling. Nor the ^{238}U content of the soil nor the concentration in the outside air indicates an extra contribution from the soil. The measures to avoid heat losses have led to a very well closed off crawl space. This property combined with a wooden floor between the crawl space and the living room may likely account for the high values in this dwelling.

For the measurements in Born and Maastricht only preliminary conclusions may be deduced due to the low number of dwellings. In Born no or only small differences were observed between concentrations in houses with or without soil covering (35 $Bq \cdot m^{-3}$), this may be due to the well sealed ground level floor. In the crawl spaces the lowest value (≥90 $Bq\ m^{-3}$) was found in the one dwelling with only an aerated concrete slab.

About twice this value was observed in the two crawl spaces with an aerated concrete slab on a polyethylene foil. In one of the dwellings where the soil was not covered a value of ≈300 $Bq \cdot m^{-3}$ was measured, in the other dwelling the crawl space was not accessible. At first sight one would conclude that coverage of the soil has a clear effect on the radon concentration in the crawl space; the factor of five difference as observed in the crawl spaces in NW-Maastricht, however, indicates that the differences could be due to differences in ventilation of the crawl space or soil conditions of the dwellings. Either more dwellings or measurements before and after introduction of soil coverage are necessary to come to firm conclusions.

The results for the dwellings in Maastricht are presented in fig. 5 for sets of four and five "identical" dwellings in NW-and SE-Maastricht, respectively. Despite the low number of dwellings one observes that in NW-Maastricht the concentration in the crawl space, C_k, varies a factor five between the dwellings, indicating large local differences in ventilation of the crawl space or in soil properties; in SE-Maastricht C_k is grouped between 600 and 800 $Bq \cdot m^{-3}$. The increase of the concentration in the living room, C_w, with increasing concentrations in the crawl space in NW-Maastricht is consistent with a ventilation in which 4% of the air enters via the crawl space. In SE-Maastricht C_w decreases with increasing C_k. Such a pattern is consistent with a more or less constant ventilation rate of the crawl space and small differences between the dwellings in the leakage area in the floor separating living room and crawl space.

The results presented above indicate that these two sets of dwellings may represent test cases for better understanding the infiltration of radon in dwellings. Measurements of ventilation patterns in these dwellings to check the preliminary suggestions may enhance the knowledge on this topic.

5. Radon and ground water

The diffusion length in water is about two orders of magnitude smaller than in non-watersaturated sand. This means that for situations in which the groundwater level is less than a few diffusion lengths in sand under the surface the radon concentration in the soil gas near the surface, and hence the exhalation rate is influenced by the thickness of the layer of non-saturated soil. Diffusion model calculations show an increase by about a factor of 5 for the radon concentration in a crawl

space if the groundwater level is lovered from 10 cm belov the surface to 50 cm.

Experiments, near a test house were many radon related experiments have been and will be carried out, were conducted in which groundwater levels and rainfall were recorded daily and radon concentrations were measured in a pipe, closed on the top side and placed into a hole in the soil with a depth of 40 cm. Time-averaging radon cups were placed in the pipe for about one week.

The results indicate that there is a correlation between the averaged radon concentration and the average groundwater level in the previous week. Fig. 6 shows the data collected in the period May to October. The data shov a good correlation, especially if one realises that due to build up and decay of radon, different radon concentrations are to be expected for situations with the same average groundwater level over the week but with groundwater level increasing or decreasing.

Based on these results it is expected that radon concentrations in dvellings will increase if the groundwater table is lovered, e.g by drainage to reduce moisture problems.

6. Conclusions

From this report it can be concluded that the objectives are well met. Especially progress has been made in understanding the mechanisms influencing the radon exhalation detection. The results indicate that solving unforeseen difficulties have led to a better description of processes influencing the collection of radon decay products in the exhalation meter. Moreover, the first set of exhalation measurements of building materials and soil indicates a proper functioning of the instrument and underlines the improvements in the design.

Experiments of charcoal canisters to reduce the sensitivity of the collection efficiency have not yet been successful. Sealing with humidity resistant foils have resulted in a too large reduction in the radon diffusion constant. After consulting the Program Officer it was decided to carry out a limited number of further tests. If again no progress is obtained this part of the program will be terminated with the conclusion that activated charcoal canisters are only to be used as first indicators for radon concentrations. If needed a monitor more precise measurements may be carried out with the continuous radon monitor, which is being developed.

Measurements of radon concentrations in parts of dwellings with high concentrations confirm the important role of the crawl space for the indoor radon levels. In a number of cases an inverse correlation was found between levels in the living room and the crawl space. This correlation is consistent with a larger ventilation of the crawl space via the dwelling.

Measurements to reduce the influx of moisture to the dwelling by covering the soil with foil and/or concrete, combined with sealing openings in the floor between crawl space and living room, indicate some reduction in the radon concentration in the crawl space but, probably because of well sealed floors, they did not show significant lower radon concentrations in the living room of comparable dwellings with no covered crawl space soil. More detailed studies are necessary to evaluate the effectiveness of these countermeasures for radon reduction.

First experiments and model calculations indicate high radon concentrations ($\cong 10^4$ Bq·m^{-3}) in soil gas of soil with a low radium content. The radon levels show a good correlation with changes in groundwater level. In the Dutch situation often drainage is applied if groundwater levels are too high (e.g. flooded crawl spaces). As far as radon is concerned high groundwater levels reduce the exposure to radon and lowering the groundwater levels without appropriate countermeasures is thus expected to lead to enhanced radon levels in dwellings.

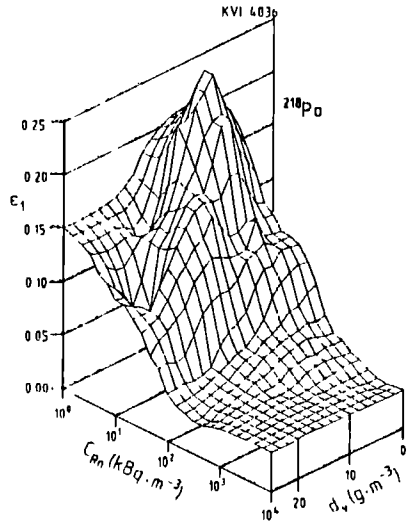
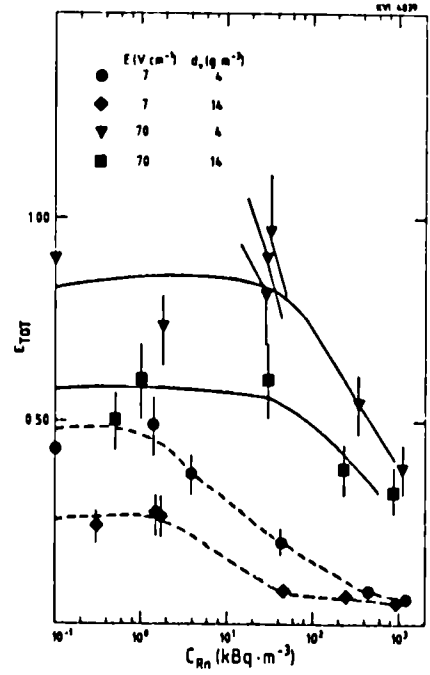


Fig. 1. Three-dimensional representation of the collection efficiency for ^{218}Po at V_{col} = 100 V as function of the radon concentration, C, and the absolute humidity, d_v.

Fig. 2. Total collection efficiency, ε_{tot}, for ^{218}Po as function of the radon concentration, C, at V_{cal} = 100 and 1200 V at T = 20°C and at low and high relative humidity.



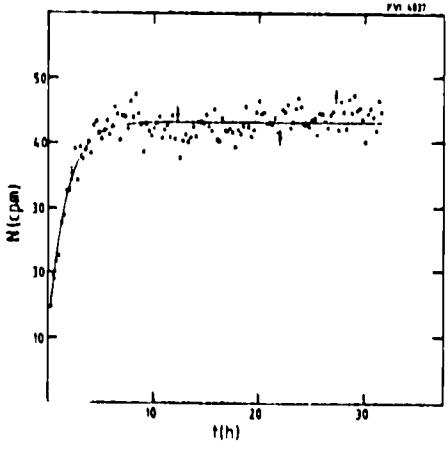


Fig. 3. Growth curve for a gypsum block placed on a table.

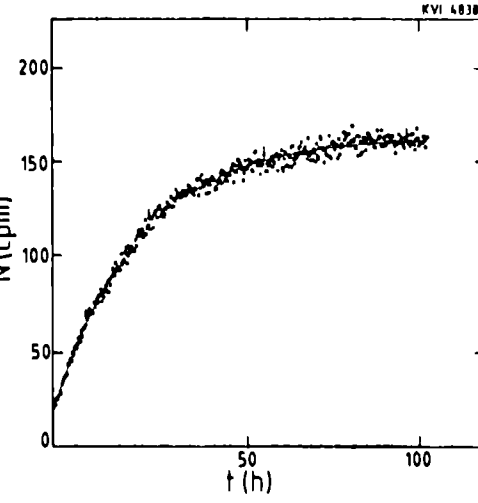


Fig. 4. Growth curve for a concrete floor. Notice the difference in time scale with fig. 3.

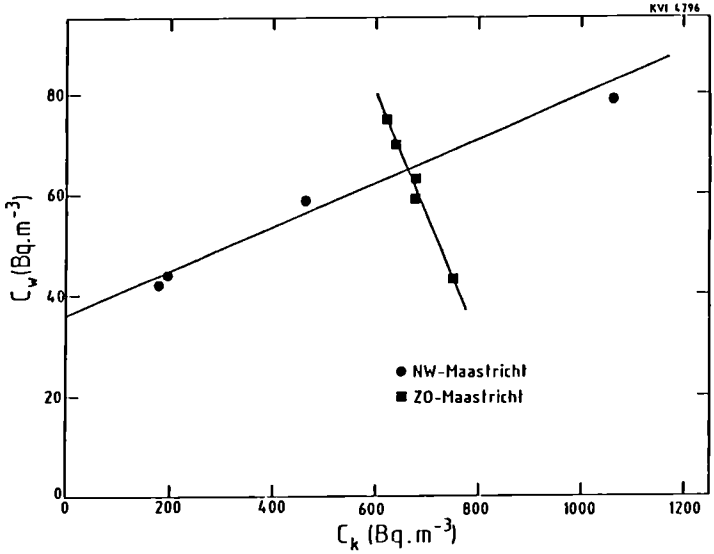


Fig. 5. Correlation between the radon concentrations in the living room and the crawl space, C_w and C_k, for two groups of identical dwellings in NW and SE Maastricht.

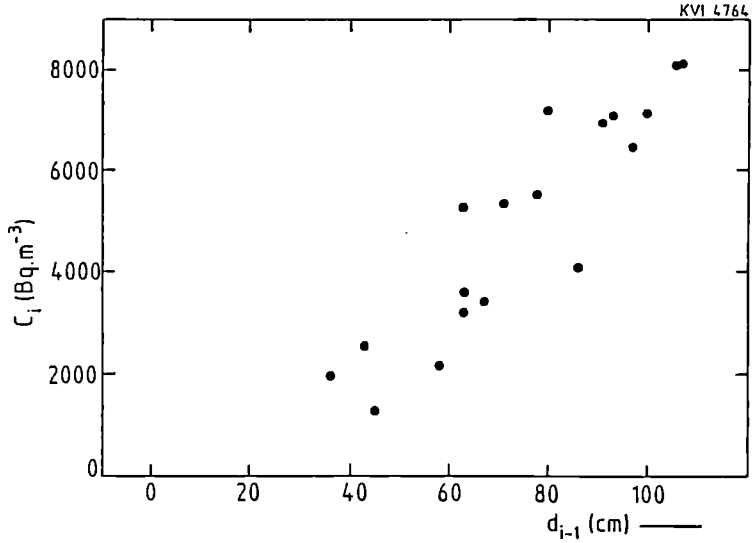


Fig. 6. Correlation between average ground water level in week i-1 and the time averaged radon concentration in week i, measured in the soil at a depth of 40 cm in a pipe, sealed at the top.

IV. Objectives for the next reporting period:

- Measurements of exhalation rates of walls, floors and soil in a number of test dwellings;
- Investigating the variability of radon exhalation by soil;
- Modelling "static" radon concentrations in terms of sources and average flows;
- Modelling radon concentrations in crawl spaces as function of ground water level, ventilation rate and soil properties
- Conclusion of the activated charcoal cannister investigation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Drs. J.G. Ackers, RD-TNO, Arnhem, the Netherlands

VI. Publications:

F.J. Aldenkamp, L.W. Put and R.J. de Meijer, "Aspects of an instrument for in situ measurements of radon exhalation rates", Environmental International 14(1988)

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: B16-F-210-NL

College van Bestuur der
Rijksuniversiteit Groningen
Postbus 72
NL-9700 AB Groningen

Head(s) of research team(s) [name(s) and address(es)].

Prof. Dr. R.H. Siemssen
Kernfysisch Versneller Instituut
Zernikelaan 25
NL-9747 AA Groningen

Telephone number (50) 63.36.00

Title of the research contract.

Measurements on, and control of infiltration of radon into dwellings.

List of projects:

1. Measurements on, and control of infiltration of radon into dwellings.

Title of the project no.:

Monitoring short term variations of indoor radon concentrations and related variables.

Head(s) of project:

L.W. Put

Scientific staff:

R.J. de Meijer
L.W. Put
P. Stoop

I. Objectives of the project:

- Improve knowledge and understanding of mechanism leading to infiltration of radon in dwellings;
- Investigate role of advective transport in soil and dwellings by studying short-term radon concentrations in correlation with relevant physical parameters;
- Deduce from (auto)correlations input for model studies;
- Deduce cost-effective countermeasures.

II. Objectives for the reporting period:

- Assembling and testing of the continuous radon-concentration monitor;
- Installation of equipment in test houses and start of measurements;
- Continue with modelling.

III. Progress achieved:

1. Continuous radon meter

1.1 Methodology

A new monitor for fast measurements of low-level radon concentrations has been developed, based on the flow-through scintillation cell. To enhance an optimum efficiency for radon, a segmentation of the cylindrical cells has been applied, which divides the volume into tubes that are not wider than 4 cm. This monitor has been designed for a measuring time of 15 to 30 minutes. For the lowest radon concentrations, a detection limit of 5 Bq/m^3 is required, in which case probably two of these monitors are needed.

1.2. Results

Using a 4 by 4 cm rectangular tube, ZnS(Ag) from several suppliers has been tested for its light emission and reflecting properties. It has been found that independent of the fabric, the light output of every 7 cm tube length added, decreases by a factor of 2. This fact restricts the tube length to a maximum of about 30 cm. With this experience, two cells have been constructed; one with a volume of 6 ℓ and one with a volume of 3 ℓ.

The 6 ℓ cell consists of three coaxial cylinders with diameters of 4, 11 and 19 cm. The outer two cylinders are divided by radial planes into small tubes. All surfaces inside the cell except the window facing the photomultiplier are covered with a mylar foil coated on one side with ZnS(Ag). A parabolic light guide has been constructed to focus the light onto a 5 cm diameter photomultiplier.

The 3 ℓ cell consists of a single cylinder with a diameter of 11 cm, not divided into smaller tubes. The cell is coupled to a 7.5 cm photomultiplier without a light guide. This cell has been made primarily made as a reference and testing model.

The photon detection efficiency of each setup has been calibrated with the following method: Only a fraction of the light output of a 0.5 inch NaI crystal irradiated by the γ-rays from a ^{137}Cs source is admitted to the photomultiplier. If the number of photo-electrons produced in the photocathode by this light is small enough, the value of this number can be deduced from its statistical variance. The number of photons in the detected light follows from the number of photo-electrons and the listed quantum efficiency of the photomultiplier. This method has proven

very useful in tracing the origin of light losses and measuring the magnitude of background signals.

The first experiments with the 6 ℓ model showed a signal to background ratio about 50 times lower than necessary for the intended detection limit. This unfavourable ratio is caused by Cerenkov light produced by cosmic rays in the light guide combined with a reduction by a factor of 14 of the light intensity in the light guide.

With the 3 ℓ cell (without light guide) a much better signal to background ratio was obtained. Measurements of radon in combination with a calibrated Lucas flask lead to an estimated detection limit of 20 Bq/m^3.

1.3. New developments

The above experiences with the cosmic-ray background have led to some adjustments in the design. Presently a 3.4 ℓ cell is being constructed consisting of two coaxial cylinders with diameters of 13 and 4 cm and eight radial planes. A 13 cm diameter photomultiplier is mounted on this cell so there will be no need for a lightguide. The estimated detection limit of this unit will be 10 Bq/m^3. Parallel to this development we investigate the possibility of coincidence measurements to reduce the Cerenkov background for the 6 ℓ cell. This step becomes necessary since recently the factory producing 19 cm diameter photomultipliers has stopped the production. Since all other preparations (sensors for various physical parameters as well as data-taking equipment are already present) are proceeding as foreseen we anticipate to start the measurements in the test house in March 1989.

2. Pressure difference measurements.

In preparation of the correlation measurements, experiments in one of the test dwelling have been carried out with a number of pressure transducers. The main purpose of the experiments was to establish the average pressure differences and the variations to be expected.

The pressure in the crawl space was at the average 2.5 Pa higher than in the above situated living room. Due to variations in wind speed fast variations in pressure occur, causing differences up to 5 and sometimes even 10 Pa. Opening and lifting the open fire raised the average difference to 7.5 Pa with variations up to 15 Pa. Opening a door at the wind side brings the living room at a higher pressure than the crawl space (1 Pa).

Pressure differences between living room and outside air showed a lower average pressure (1.5 Pa) with fluctuations of about 10 Pa in both directions. Opening doors on the wind side changes the situation to overpressure in the living room with even larger fluctuations.

Ventilation measurements, carried out several years ago in this dwelling, revealed the transparency C for separation walls and the value of the exponent n in the relation between the magnitude of the air current, q, and the pressure difference Δp:

$$q = C \times \Delta p^{1/n}.$$

The ratio of the radon concentrations in the living room and the crawl space was estimated from this equation by substituting the values of C and and n, and the average values of Δp and simplifying the air currents in the dwelling to a flow from the crawl space and one to the outside. Ignoring the low concentration in the outside air and the production of radon in the building materials of the dwelling, the estimate of 0.5 is close to experimental values measured in the period 1982-1987. It therefore may be concluded that the estimates have the correct order of magnitude. Correlation measurements foreseen in the beginning of 1989 will probably allow a more detailed comparison.

3. Model calculations

A model has been developed which describes radon concentrations as a function of time for a multi-room dwelling. The model requires as input air currents and initial concentrations and incorporates source and loss terms. It should in principle be applicable to radon as well as moisture transport. A computer program has been written on the basis of this model and has been tested for a number of cases. The results of some simple cases have been found to agree with the results of a similar program by J. G. Ackers.

As a first experimental step the pressure differences directly across the leakage areas will be measured and converted into air currents. The predictions made by the model from these air currents can be checked with radon measurements to investigate the parameters affecting radon transport. In a later phase attempts can be made to convert wind vectors to pressure differences in order to give the model a more general predictive capability.

4. Discussion

The development of the radon monitor has been slower than anticipated, and at the moment it is not sure whether the goal to reach the detection limit of 5 Bq/m^3 with a measuring time of 30 minutes be achieved before measurements in the test house are started. A detection limit of 10 Bq/m^3 in a single monitor, however will probably be available before March 1989.

Preliminary pressure difference measurements indicate that air currents in the test dwelling are such that in the living room half of the air current enters via the crawl space. It is expected that correlation measurements, combined with transport model calculations will provide insight in the possibilities to reduce the radon influx.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-116-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Stather
Biomedical Effects Department
NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: (235) 83.16.00

Title of the research contract:

Procedures to assess intakes of radionuclides from samples of airborne radioactivity and statistical studies of radiation risk.

List of projects:

1. Plate-out of radon daughter aerosols in domestic and mine environments.
2. Deposition of hygroscopic aerosols in humidified branched airways.
3. Application of CR-39 track-etch detectors to low background counting and particle sizing of air samples.
4. Modelling radiation risk in populations exposed to high doses.
5. Assessments of data from populations exposed to low doses of radiation.

A collaborative study was carried out with the Isotopenlaboratorium der Georg-August-Universität, Göttingen and the Nuclear Physics Laboratory, Gent State University. The exercise was to intercompare methods of determining the size distribution of radon daughters. Twenty-one measurements were performed in a house situated in North Bavaria, which had naturally elevated radon concentrations. Aerosol conditions were changed by generating aerosols with a propane burner, candles and cigarettes. Results obtained with the NRPB multichannel diffusion battery are shown in Table 3.

Table 3: Characteristics of airborne potential alpha-energy in a test house with various aerosol conditions

Aerosol type	Unattached		Attached	
	f_p	AMD, nm	AMD, nm	GSD
Natural	0.07	3.5	200	2.1
Propane burner	0.16	3.5	190	2.1
Candle	0.1	4.0	55	3.1
Cigarette	0.01	3.7	200	2.2

Measurements were made of other relevant parameters during the period of the exercise and these included ventilation rate, ambient aerosol concentration, size distribution of the ambient aerosol, radon and radon daughter concentrations. The full results of this exercise will be the subject of a CEC report by the participants (Reineking et al.).

References

Chu K-D, Hopke P K, Knutson E O, Tu K-W and Holub R F. (1986). Induction of an ultrafine aerosol by radon radiolysis. IN Radon and its Decay Products. Proceedings of 191st Meeting of the American Chemical Society, New York, 13-18 April, 1986.

Reineking A, Strong J C, Vanmarcke H and Van Dingenen R. Intercomparison of methods for investigating the physical characteristics of radon decay products in the indoor environment. CEC report (in press).

IV. Objectives for the next reporting period:

The multichannel diffusion battery will be modified so that the size range of the instrument will be extended to cover particle diameters down to 1 nm: this will allow better estimates to be made of the unattached activity particle size distribution. Further measurements will be made in a representative range of dwellings and domestic conditions and in a selection of metal mines. This will provide more reliable data for the application of the model linking exposure and dose described in the previous progress report.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr A Reineking, Isotopenlaboratorium der Georg-August-Universität, Burckhardtweg 2, D-3400 Göttingen, F.R.G.

Dr H Vanmarcke, Nuclear Physics Laboratory, Gent State University, Proeftuinstraat 86, B-9000 Gent, Belgium.

VI. Publications:

Strong J C. Radon daughter aerosols in dwellings. Radiological Protection Bulletin No. 94, July 1988.

Strong J C. The size of the attached and unattached radon daughters in room air. Presented at the European Aerosol Conference organised by GAeF, Lund, September, 1988.

IV. Objectives for the next reporting period

To investigate the spatial deposition of particles in the nasal cast within the size range 50 to 250 nm diameter and with unattached lead-212. To measure the penetration through the nasal cast of radon daughters in the size range 1 to 5 nm diameter. To estimate the doses to the nasal tissues of adult persons and to extrapolate to persons of younger age.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr D L Swift, Associate Professor, Department of Environmental Medicine, Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA.

Dr J E Agnew, Department of Medical Physics, The Royal Free Hospital, Pond Street, Hampstead, London NW3 2QG.

VI. Publications:

Strong J C and Agnew J E. The particle size distribution of Technegas and its influence on regional lung deposition". Nuclear Medicine Communications (in press).

Title of the project no.: 3

Application of CR-39 etched track detectors to low background counting and particle sizing of air samples.

Head(s) of project:

J C H Miles

Scientific staff:

L McDonough, R A Algar

I. Objectives of the project:

To develop the technique of alpha-particle registration on CR-39 to the stage where it becomes suitable for routine assay of long-lived alpha activity collected on personal air sampler filters.

II. Objectives for the reporting period:

To develop and test software for automatic scanning and assessment o etched-track autoradiography. To test the software on autoradiographs of personal air sampler filters.

Title of the project no.: 4

Modelling radiation risk in populations exposed to high doses.

Head(s) of project:

Dr. C.R. Muirhead

Scientific staff:

Mr. A.M. Ball

I. Objectives of the project:

To model the pattern of radiation-induced cancers in populations exposed to high doses of radiation. In particular, to model the effect of dose, age and temporal factors on the radiation-related cancer risk.

II. Objectives for the reporting period:

To study variation with age and time in the radiation-induced cancer risk among U.K. ankylosing spondylitis patients given x-ray therapy.

To develop cancer risk estimates applicable to a U.K. population on the basis of recently published information concerning the Japanese atomic bomb survivors and using other relevant studies.

III. Progress achieved:

Ankylosing Spondylitics Patients

Analysis of age and time trends in radiation-induced cancer risks has been undertaken using data from the latest follow-up of U.K. ankylosing spondylitis patients who received x-ray therapy (Darby et al, Br. J. Cancer, 1987). For the grouping of all cancers other than leukaemia and colon cancer, the relative risk at a given time since exposure was found to decrease with increasing age at exposure, in line with results from the Japanese atomic bomb survivors (Shimizu et al, RERF TR5-88, 1988). However, the trends in risk with time since exposure were such that both the relative and absolute excess risk began to tail off about 20 years following exposure. Thus different methods for projecting risks beyond the current period of follow-up yielded similar lifetime risks on the basis of these data. The above result contrasts with the most recent follow-up of the Japanese atomic bomb survivors (Shimizu et al, 1988), where the absolute excess risk of solid cancers is continuing to increase with increasing time since exposure and the relative risk is approximately constant up to 40 years post exposure. However, a tailing-off in the radiation-induced risk as in the spondylitics has also been seen, for example, in lung cancer among uranium miners and bone cancer in U.S. radium luminisers (BEIR IV, 1988).

Risk Estimates Developed from the 1988 UNSCEAR Report

In the wake of recent publications by the Radiation Effects Research Foundation (RERF) in Japan on cancer risks among the A-bomb survivors based on the new DS86 dosimetry system and of the 1988 UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) review of risk estimates, health effects models applicable to a UK population have been developed (Stather et al, NRPB-R226, 1988). For cancer, risk estimates were derived mainly on the basis of the Japanese data, but using other studies where these were more informative for particular cancers (eg. using data on radium luminisers in the case of bone cancer, and on patients given x-ray treatment for enlarged thymus in the case of thyroid cancer). Owing to the uncertainty in the prediction of lifetime risks as described above, two sets of risk estimates were calculated for solid tumours; one set based on the follow-up of the A-bomb survivors to date (40 years post exposure) and the other set based on the assumption that relative cancer risks will remain constant until the end of life. Based on an analysis of radiation-induced cancer rates and baseline rates for individual cancer types in the Japanese survivors and UK ankylosing spondylitics patients, it was decided for solid tumours to transfer risk coefficients derived from the former population to a UK population on the basis of relative risk, since this parameter was more stable across populations than absolute risk. In order to extrapolate to low doses and low dose rates, a dose rate effectiveness factor (DREF) of 3 was used in most cases, as suggested by a review of animal studies; however, the DREF was taken equal to 2 for breast cancer.

Table 1 shows risk estimates applicable to a UK population of all ages and both sexes. The total fatal cancer risk at low doses and dose rates was estimated as 1.4-4.5% Gy^{-1} (low LET). The range of uncertainty due to lack of information from current cohort studies on risks over a complete lifetime is greatest for those exposed in childhood. For a working population aged 20-64 years at exposure, the range of uncertainty is less than for the whole population and estimates of 1.8-3.4% Gy^{-1} (low LET) were obtained for all cancers; see Table 2.

Table 2

Estimated excess cancer risks in a UK working population (both sexes) exposed to low LET radiation at ages 20–64 years

Cancer type	Fatal cancer risks, 10^{-2} Gy^{-1} (low LET)				ICRP 1977
	Lifetime projection		Risk observed to date[i]		
	HDR[g]	LDR[h]	HDR	LDR	
Leukaemia[a]	1.01	.34	1.01	.34	.2
Breast[b]	.79	.40	.51	.26	.25
Lung[a]	2.09	.70	1.64	.55	.2
Thyroid[c]	.05	.017	.03	.01	.05
Bone[d]	.15	.05	.15	.05	.05
Liver[e]	.45	.15	.23	.08	.1
LLI/colon[a]	1.21	.40	.56	.19	.1
Stomach[a]	.59	.20	.19	.06	.1
Remainder[a,f]	3.33	1.10	.88	.28	.2
Total[a]	9.67[j]	3.4	5.20	1.82	1.25

Notes:

(a) based on Japanese A-bomb survivors

(b) based on studies in western women

(c) from NCRP Report No 80 (1985)

(d) based on radium luminisers

(e) based on Thorotrast cases (BEIR IV, 1988)

(f) by difference

(g) high dose rate (> 100 mGy d^{-1})

(h) low dose rate (< 100 mGy d^{-1})
DREF: 2 for breast, 3 for other cancers

(i) based on period of follow-up of Japanese population to date (40 years)

(j) corresponds to 9.67% Gy^{-1}

(k) from Stather et al (1988)

Other Studies

A tape containing data on lung cancer mortality and radon exposure (plus information on smoking) for the Colorado Plateau uranium miners has been obtained from the U.S. National Institute of Occupational Safety and Health (NIOSH). The interpretation of some of the data on this tape is currently being checked with NIOSH. Also, a floppy disk containing the full cancer data from the latest follow-up of the Japanese atomic bomb survivors, based on the new DS86 dosimetry, is awaited from RERF in Japan.

It is planned to undertake extensive modelling of radiation-induced cancer risks using both these data sets.

IV. Objectives for the next reporting period:

To obtain data from the latest follow-up of the Japanese atomic bomb survivors and to analyse trends in cancer risk with age, time and dose. To perform similar types of analyses on data for the Colorado uranium miners, including an examination of the joint effect of smoking and radon exposure. To compare the results with those for the U.K. ankylosing spondylitis patients given x-ray therapy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Imperial Cancer Reseach Fund Cancer Epidemiology and Clinical Trials Unit, University of Oxford, Radcliffe Infirmary, Oxford, OX2 6HE, UK. (Collaborating research worker - Dr. S.C. Darby).

VI. Publications:

Muirhead, C.R. and Darby, S.C., Distinguishing relative and absolute risk models for radiation-induced cancers. IN Health Effects of Low Dose Ionisnig Radiation - Recent Advances and their Implications, pp45-50. London, British Nuclear Energy Society (1988).

Muirhead, C.R. and Darby, S.C., Relative and absolute risk models for cancer mortality in ankylosing spondylitis patients. In Proceedings 14th L.H. Gray Conference, Oxford, September 1988 (to appear).

Stather, J.W., Muirhead, C.R., Edwards, A.A., Harrison, J.D., Lloyd, D.C. and Wood, N.R., Health effects models developed from the 1988 UNSCEAR report. Chilton, NRPB-R226 (London, HMSO) (1988).

Title of the project no.: 5

Assessments of data from populations exposed to low doses of radiation.

Head(s) of project:

Dr. C.R. Muirhead

Scientific staff:

Mr. A.M. Ball

I. Objectives of the project:

To develop and refine techniques for analysing epidemiological data on occupational and other populations exposed to low levels of ionising radiation. To develop and validate statistical software for use in the study of populations exposed occupationally to radiation.

II. Objectives for the reporting period:

To continue the refinement and validation of the computer program written to analyse occupational data.

To review data from studies on irradiation in utero and subsequent cancer risks.

To continue to assess reports of cancer clusters near nuclear installations.

III. Progress achieved:

Software for Analysing Occupational Data

The computer program ARFAR (At Risk For Any Reason), written at NRPB to assist in the analysis of data from studies of those exposed occupationally to ionising radiation, has been refined further. The purpose of the program is to allow person-years-at-risk to be stratified by cumulative radiation dose, as well as by age, calendar period, etc., so that an internal test for a trend in cancer rates with dose can be performed. It is intended that the program be used in the analysis of the National Registry for Radiation Workers (NRRW).

One refinement now allows more general latency distributions for risk following exposure to be accommodated. In particular, rather than assuming that a radiation-induced cancer risk suddenly commences at a specific time following exposure, ARFAR can now stratify person-years on the basis of a model for which the risk increases linearly with time until it reaches a plateau. While the former model is routinely used in analyses of occupational studies and so will be used in the first analysis of the NRRW, the latter model allows for greater flexibility and for assessing the sensitivity of the latency assumptions.

Another modification to ARFAR has been made that will reduce considerably the computing time arising from multiple runs of the program. This consideration is particularly important in relation to the NRRW, where large numbers of runs will be required. The saving in computing time has been achieved by producing a separate version of ARFAR in which only tables of numbers of deaths from a given cause are produced. Thus only one run of the original version of ARFAR is required to obtain tables of person-years, while the modified version can be run repeatedly for different causes of deaths.

Work is currently in progress on a program to calculate median doses within dose groups, such that trends in mortality can be examined in relation to these median doses.

ARFAR has been made available to other research workers concerned with epidemiological studies and is available on request.

Cancer Risks following Irradiation In Utero

Discussions have taken place with members of the Department of Social Medicine at the University of Birmingham concerning a paper they had published at the end of 1987 on childhood cancer (Knox et al, J. Soc. Radiol. Prot., 7, 177-189). This paper contained a re-analysis of data on pre-natal x-rays and childhood cancer obtained from the Oxford Survey of Childhood Cancers (OSCC). In particular, it was suggested in the paper that adjusting in the analysis for the effects of both drugs administered to the mother and maternal illnesses during pregnancy increased the excess relative cancer risk associated with pre-natal x-rays by more than a factor of 2.

As a result of discussions with the authors and further analyses of the data it was found that an error had been made in their calculations; in particular, the cohort-specific cancer risks and the relevant years of birth had been mis-matched. When these were aligned correctly it was found that the relative risks were similar to those obtained in earlier analyses

of this study giving a relative risk of about 1.4. A joint letter with the authors to the Editor of the above journal pointing out the error and providing revised risk estimates is in preparation.

As part of the development of health effects models in the light of the 1988 UNSCEAR report, data on cancer risks following irradiation *in utero* have been reviewed (Stather *et al*, NRPB-R226, 1988). These consisted of data from the OSCC, from other studies of pre-natal x-rays and childhood cancer (for example, that by MacMahon in the N.E. United States), and from the Japanese atomic bomb survivors. Based on the OSCC and estimates of doses from pre-natal x-rays given by UNSCEAR (1970), a risk of cancer incidence following *in utero* exposure of 6% Gy^{-1} was adopted (low LET), with values of 2.5% Gy^{-1} for leukaemia and 3.5% Gy^{-1} for other cancers. Half of these cancers were assumed to be fatal.

Assessing Cancer Clusters in Relation to Nuclear Installations

Some reports suggesting the presence of raised levels of childhood leukaemia near UK nuclear installations such as CEGB Hinkley Point have been studied. As is usual in such reports, there are problems in deciding how to choose boundaries for the study area, time period, and age and disease grouping, as well as in the choice of control group. It is important to assess how sensitive the results are with respect to these choices when attempting to interpret the reports.

In the case of leukaemia clusters in the region of Hinkley Point epidemiological data for the period prior to start up of the plant indicated that a high leukaemia incidence was already prevalent in the area.

On the methodological side, assistance is being provided in organising a Royal Statistical Society Meeting on Cancer near Nuclear Installations, to be held in London in May 1989. The aim of this meeting is to encourage statisticians who have not previously dealt with this topic to apply their expertise in this area.

IV. Objectives for the next reporting period:

To continue to refine and validate the computer program ARFAR so that it can be used to analyse data from the National Registry for Radiation Workers.

To study cancer risks in relation to in utero exposure further, based on original data sets if any become available.

To make further study of statistical issues concerning the geographical pattern of cancer in relation to nuclear installations.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Institut für Strahlenschutz, Gesellschaft für Strahlen- und Umweltforschung,
Ingolstädter Landstrasse 1, D8042 Neuherberg, Federal Republic of Germany.

VI. Publications:

Stather, J.W., Muirhead, C.R., Edwards, A.A., Harrison, J.D., Lloyd, D.C. and Wood, N.R., Health effects models developed from the 1988 UNSCEAR report. Chilton, NRPB-R226 (London, HMSO) (1988).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-213-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.W. Stather
Biomedical Effects Department
NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Telephone number: 0235-831600

Title of the research contract:

The risks of radiation work: analysis of registry data.

List of projects:

1. The risks of radiation work: analysis of registry data.

Title of the project no.: 1

The risks of radiation work : analysis of registry data

Head(s) of project:

Dr. G.M. Kendall
Dr. C.R. Muirhead

Scientific staff:

Mrs. J.A. O'Hagan
Miss S.M. Walker
Mr. A.M. Ball
Miss S. Brooks

I. Objectives of the project:

To determine by a follow-up study whether there is any evidence of differences in the cause of and age at death of workers exposed to different levels of radiation. To estimate the magnitude and place bounds on any radiation-attributable risk. To compare the mortality rates of radiation workers with those of other industrial groups and with national mortality rates.

II. Objectives for the reporting period:

To expand and improve the registry data base. To plan the analysis of data.

III. Progress achieved:

As noted in previous reports, perhaps the main problem facing the National Registry for Radiation Workers (NRRW) is that there has been a substantial fraction of the study population whose records could not be flagged with the National Health Service Central Register. This is essential if follow-up information (eg. date and cause of death) is to be obtained. At the time that the third supplement to the Protocol (NRPB-R219) was published over 11,000 individuals, 12% of the total, could not be flagged in this way; see Tables 1 and 2. Considerable efforts have been made and the total number of records which cannot be flagged has dropped by about half. New ways of tackling this problem have been identified which should result in a further substantial drop in the number not flagged. With an increase in the number of individuals enrolled, the percentage flagged is now (26.1.89) 90%.

There have also been problems in obtaining data from one large employer of radiation workers. A number of actions have been undertaken to co-ordinate the transfer of the various components of missing data and present indications are that there should be a substantial improvement in the early part of 1989.

A report describing the plans for the analysis of the NRRW has been circulated to a number of eminent epidemiologists for comment. A revised version will shortly be published as NRPB-M149.

Regarding the analysis, some study has been made of the likely effect of dose record keeping practices on tests for trend in disease rates with dose. In particular, systematic errors in cumulative recorded doses arise through the subtraction of values from annual doses as a consequence of recording levels and as a means of allowing for threshold doses. It appears that allowing for length of time monitored in the analysis may reduce the effect of such dose measurement errors. A possible alternative, if data on time monitored are not available, is to allow for length of employment. This topic will be studied further.

Table 1: Status of Study Population, as given in NRPB-R219

Study population	Number enrolled	Positive refusals	Individuals of uncertain status
97,437	94,845 (97%)	1752 (1.8%)	840 (0.9%)

Number of individuals enrolled on NRRW	Number flagged	Number not flaggable
94845	83454 (88%)	11391 (12%)

Table 2: Distribution of Doses, as given in NRPB-R219

	Lifetime dose (mSv) <10	10-50	>50	Total
No. of employments:				
Participants	52347 (55%)	26529 (28%)	15969 (17%)	94845 (100%)
Non-participants	(49%)	(34%)	(17%)	(100%)
Collective dose for participants (man Sv)	133 (4%)	577 (19%)	2266 (76%)	2976 (100%)

IV. Objectives for the next reporting period:

To attempt to complete the data base up to the required date of follow-up and undertake data validation.

To commence the first analysis of the data base.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Imperial Cancer Research Fund Cancer Epidemiology and Clinical Trials Unit, University of Oxford, Radcliffe Infirmary, Oxford, OX2 6HE, UK. (Consultants Dr. S.C. Darby and Sir Richard Doll).

VI. Publications:

(1) Kendall, G.M., O'Hagan, J.A., Rees, S., Walker, S.M. and Muirhead, C.R. "Summary of data held by the National Registry for Radiation Workers". Chilton: NRPB-R219 (1988).

(2) Ennis, J.R., Kendall, G.M. and Muirhead, C.R. "Proposals for analysis of the data held by the National Registry for Radiation Workers". Chilton: NRPB-M149 (to be published).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Commissariat à l'Energie
Atomique, CEA
IPSN
B.P. n° 6
F-92265 Fontenay-aux-Roses

Contract no.: BI6-F-122-F

Head(s) of research team(s) [name(s) and address(es)]:

M. G. Uzzan
IPSN-DPS
CEA-CEN de Fontenay-aux-Roses
B.P. n° 6
F-92265 Fontenay-aux-Roses

Telephone number: (1) 654.71.39

Title of the research contract:

Consequences of irradiation of population and workers.

List of projects:

1. Assessment of industrial irradiation.
2. Assessment of the objective detriment and of its coat, in relation to economic considerations.
3. Assessment of the subjective dimension of the radiological detriment, in relation to sociological considerations.

Title of the project no.: 1

Evaluation des irradiations industrielles/Base de données européennes

Head(s) of project:

A. GARNIER

Scientific staff:

A. GARNIER

I. Objectives of the project:

Rassembler sous une forme harmonisée, connue sous le nom de grille européenne, des données générales de base nécessaires aux évaluations des conséquences sanitaires et socio-économiques des rejets (normaux ou accidentels) de l'industrie nucléaire, pour les populations des pays de la CEE.

II. Objectives for the reporting period:

- Répartition, dans les mailles larges (10 000 km²) de la grille européenne, de diverses données régionales complétant celles précédemment acquises.

- Unification pour l'ensemble de la grille, sans distinction de frontières nationales à l'intérieur de la Communauté, de la répartition établie d'abord pays par pays.

- Comunication des résultats et échange d'informations avec les participants du projet MARIA.

III. Progress achieved :

1. *Méthodologie*
Le cadre géographique est celui connu et déjà utilisé sous le nom de grille européenne, à larges mailles (10 000 km²) ou à mailles fines (100 km²).

Le cadre administratif dans lequel sont obtenues les données est en général celui des régions figurant dans la Nomenclature des Unités Territoriales des Statistiques Européennes, agrégées à trois niveaux : Les informations, classées selon une nomenclature uniforme, proviennent en majorité de la banque REGIO (niveau II de la NUTS, c'est-à-dire unités administratives de base, au nombre de 118 pour la Communauté). Elles sont complétées lorsqu'il y a lieu par saisie de documents statistiques (cas des nouveaux pays de la CEE).

La distribution des aires des unités territoriales dans les mailles de la grille, réalisée par méthode cartographique ou par méthode informatique, conduit à une série de coefficients de pondération. Ceux-ci permettent la répartition des quantités correspondant aux diverses rubriques en supposant qu'elle soit uniforme à l'intérieur de chaque région (ce qui est parfois contestable).

Ces opérations de saisie de données et de répartition territoriale ont dû être faites pays par pays.

La présentation finale des résultats est unifiée maille par maille, chacune de celles-ci pouvant inclure de un à trois pays.

3. *Résultats*
Les résultats fournis pour les Douze à quelques exceptions près concernent les rubriques suivantes :

- Utilisation du territoire (Unité : km²) : superficie totale, superficie boisée, superficie agricole utilisée, superficie des jardins familiaux, superficie toujours en herbe, superficie des cultures permanentes (dont vignes, oliviers), superficie des terres arables, superficie des fourrages verts. Dans le cas de l'Espagne, s'y ajoutent : superficie agricole, eaux, autres superficies, total irrigué.
- Superficies cultivées et productions végétales (Unités : km² et milliers de tonnes) : céréales (total), blé et épeautre, orge, seigle, avoine, maïs grain, riz, pommes de terre, betteraves sucrières, oléagineux, maïs fourrager, colza, tournesol, tabac.
- Effectifs du bétail (milliers de têtes) : bovins (total, vaches laitières, autres vaches), porcs (total, truies d'élevage), ovins, caprins, équidés.

- Emploi total par branches d'activités économiques (milliers d'emplois) : 17 branches d'activités, dans les secteurs : agricole et forestier, produits énergétiques et industriels (dont minerais, produits métalliques, produits chimiques, ..), bâtiment et génie civil, Services marchands et non, marchands, etc..).
- Population (milliers d'habitants) : Grèce, Espagne, Portugal, complétant la procédente distribution établie par mailles fines pour les anciens pays de la CEE.

L'année de référence choisie était 1981 pour l'Utilisation du Territoire et l'Agriculture, mais selon la disponibilité des données, on en a parfois utilisé de plus récentes (exceptionnellement de plus anciennes).

Pour les informations sur l'emploi par secteurs économiques, nouveau sujet d'intérêt, il a paru préférable d'adopter les plus récentes disponibles dans REGIO, à savoir : 1983 pour la France, 1981 pour le Portugal, 1984 pour les autres pays.

3. ***Discussion***

Les buts fixés pour la période considérée ont été atteints.

La copie des fichiers informatiques va être mise à la disposition des Communautés.

En outre, les échanges d'informations dans le cadre du projet MARIA ont facilité la réalisation, par certains participants, d'études détaillées en harmonisation avec le modèle général.

IV. Objectives for the next reporting period :

- Développement des données et des méthodes permettant l'évaluation des conséquences économiques de rejets accidentels.

- Eventuellement, amélioration de la représentativité de la répartition dans les secteurs sensibles.

V. Other research group(s) collaborating actively on this project /name(s) and address(es) / :

Wharton Economic and Financial Information Services (WEFA)
25 Rue de Ponthieu - 75 008 PARIS.

VI. Publications

- A. GARNIER - Progress in the preparation of demographic and land use data bases. In : Joint CEC/OECD (NEA) workshop on recent advances in reactor consequence assessment, Roma, Janv. 88, Report EUR 11408 EN, 345-356

- A. GARNIER et A. SAUVE - Notices descriptives et fichiers informatiques concernant
- l'utilisation du territoire français
- l'utilisation agricole du territoire de l'Angleterre et du pays de Galle (répartition par mailles de 100 km²)

- A. GARNIER - Development of data bases in the european grid model using regional data - MARIA meeting (CEC/KFK) Karlsruhe, oct. 88
- *A paraître* :
- A. GARNIER - Notice descriptive de la grille européenne et du fichier informatique concernant la répartition sur le territoire de la Communauté Européenne (mailles de 10 000 km² des résultats sur : l'utilisation du territoire, les productions agricoles (végétales et animales), la population et l'activité par secteurs économiques.

Title of the project no.:

Evaluation du détriment objectif global et de son coût en relation avec les considérations économiques - Evaluation du détriment objectif chez l'homme - Travaux post Tchernobyl.

Head(s) of project:

A. DESPRES

Scientific staff:

A. DESPRES

I. Objectives of the project:

Elaboration d'un rapport de synthèse relatif aux mesures anthropogammamétriques effectuées dans les différents pays d'Europe après l'accident de Tchernobyl.

II. Objectives for the reporting period:

Rédaction du rapport final

III. Progress achieved:

Les mesures effectuées sur l'homme ou sur les excrétats après l'accident de Tchernobyl diffèrent en chaque pays sur un certain nombre de points :

- le matériel utilisé (installations fixes ou mobiles)
- la taille des échantillons observés
- les buts poursuivis (évaluation du détriment radiologique, évaluation de la contamination interne et vérification des modèles métaboliques, comparaison avec les activités ingérées calculées à partir de la contamination de la chaîne alimentaire).

Dans tous les cas, les seuls radionucléides étudiés sont les césiums (^{134}Cs et ^{137}Cs), et l'iode 131.

Les constatations essentielles portent sur :

- la grande disparité des contaminations à l'intérieur d'une même population ; d'une façon générale, les quantités de césium incorporées sont plus grandes chez les hommes que chez les femmes, et chez les femmes que chez les enfants,
- la bonne concordance de la décroissance observée de l'activité de l'organisme après la fin de l'exposition avec celle suggérée par les modèles métaboliques,
- la surestimation systématique des activités ingérées calculées à partir de la contamination de la chaîne alimentaire,
- la cartographie qu'il est possible d'établir et regroupant l'ensemble de ces mesures est en bon accord avec la cartographie des dépôts.

IV. Objectives for the next reporting period:

Sans objet.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

TITLE OF THE PROJECT N°

Evaluation du détriment objectif global en relation avec les considérations économiques. Recherches sur l'homme.

HEAD(S) OF PROJECT
M. R. MAXIMILIEN
I.P.S.N./D.P.S.
BP n° 6
92265 FONTENAY AUX ROSES
Cedex

SCIENTIFIC STAFF

1 personne

I. OBJECTIVES OF THE PROJECT

- L'objectif de cette étude est de comparer les méthodes d'évaluation du détriment chimique avec celles utilisées dans le domaine radiologique dans une perspective d'harmonisation des pratiques et des choix en matière de gestion des risques.

II. OBJECTIVES OF THE REPORTING PERIOD

Analyser de façon comparative les méthodes d'évaluation du risque cancérigène entre métaux non ferreux et rayonnements ionisants en se basant sur les résultats de l'expérimentation animale et les effets génotoxiques

III. PROGRESS ACHIEVED

Méthode : Etablissement de profils toxicologiques pour les dérivés inorganiques du nickel (spéciation) susceptibles d'être à l'origine du risque cancérigène observé dans les raffineries :

- revue critique des résultats de l'expérimentation animale à l'aide des lignes directrices édictées par la C.E.E. pour les essais de cancérogénèse.
- revue des tests à court terme in vivo et in vitro pour mettre en évidence des indicateurs quantitatifs de l'éventuelle activité cancérigène sur une base mécaniste.

Résultats :

1. analyse de l'expérimentation animale : la spéciation du risque cancérigène des dérivés nickellifères pose des problèmes de différents ordres :

- incertitudes sur les formes physico-chimiques précises des composés nickellifères utilisés dans les essais, particulièrement dans le cas des oxydes, des carbonates et des hydroxydes.
- représentativité des voies d'administration proposées en cancérogénèse expérimentale eu égard aux modes d'exposition de l'homme : les données expérimentales sur l'inhalation sont peu nombreuses. Le recours à des voies respiratoires de substitution (intratrachéal...) reste sujet à caution car elles appliquent de fortes expositions sur des périodes de temps courtes c.a.d. sans mettre en jeu les mécanismes physiologiques de distribution et de clairance dans l'arbre respiratoire. Bien qu'ayant un intérêt bien moindre pour l'extrapolation vers l'homme, les voies parentérales (intramusculaire, intrarénale, intraveineuse, intrapéritonéale) sont les plus documentées. Au bilan, l'expérimentation animale montre l'absence d'effet cancérigène du nickel et de ses dérivés par voie orale et des résultats non probants par inhalation ; la seule exception concerne l'induction manifeste de cancers du poumon chez le rat dans une seule étude où il était soumis à une inhalation chronique de sous sulfure de nickel. En ce qui concerne les voies non représentatives les données animales montrent une action cancérigène locale, parfois importante (voie intramusculaire++) mais n'apportent pas de preuve d'une action cancérigène systémique. Si l'on considère que de fortes concentration de nickel ou de ses composés sont à l'origine des effets observés in situ, il ressort qu'on ne peut conclure pour l'homme chez lequel on a uniquement observé des cancers de la sphère respiratoire au niveau de muqueuses exposées à de fortes concentration de composés mal définis du nickel.
- insuffisance des protocoles expérimentaux (faibles effectifs, durée de suivi courtes, niveaux de doses uniques...) qui ne permet pas de conclusion définitive sur la présence ou l'absence d'effet cancérigène.

2. étude des tests à court terme : du point de vue de l'interprétation des mécanismes d'action des substances nickellifères à l'échelle cellulaire et de l'utilisation qui peut en être faite pour en comparer le potentiel d'activité cancérigène, on peut ranger les tests à court terme en 2 catégories :

- les tests d'action clastogène sur différents systèmes de cellules mammifères dont l'inventaire montre qu'ils sont hétérogènes tant sur le plan de la gamme des substances testées, que sur celui des protocoles, des mesures et des modes d'expression des résultats.
- les tests de transformation de cellules de hamster chinois qui permettent de mettre en regard des indicateurs d'incorporation cellulaire

(% de phagocytose, cytotoxité) et des indicateurs d'effet(transformation cellulaire). Le bilan des données disponibles dans la littérature indique un parallélisme entre phagocytose des composés insolubles et la transformation des cellules du même système cellulaire ou de celles d'un système voisin. Par ailleurs, l'identité des potentiels de transformation de divers composés à niveau de cytotoxicité égal semble conforter l'hypothèse de l'ion, mais elle n'a été vérifiée que dans un seul système.
Ces observations demandent à être confirmées expérimentalement au moyen de méthodes standardisées faisant appel à une seule souche cellulaire (les interprétations proposées étant habituellement issues de résultats portant sur plusieurs souches), un protocole unique (durée d'exposition et d'incubation fixées), un observateur unique (évaluation semi quantitative). Dans une perspective plus lointaine, l'utilisation de systèmes cellulaires issues d'espèces différentes pourra contribuer à valider la procédure d'extrapolation vers l'homme sur une base mécaniste. En ce sens, la démarche est analogue à celle utilisée dans le cas des rayonnements ionisants.

VOIES	ESPECES	Ni métal		Ni_3S_2		NiS		Ni ox		$NiCO_3$		$Ni(OH)_2$		$NiSO_4$		$NiCl_2$		$Ni(CO)_4$	
		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Inh	Rat		4	1				2	4						1			4	
	Souris			1															
	Cobaye	1																	
	Hamster		1					1	3										
Itr	Rat	1		2	1			1											
	Souris			1															
	Hamster	1	1		1			1											
Int nas	Rat		1																
Int bro	Rat	1																	
Int plo	Rat	2						1											
	Souris		1																
Oral	Rat														3		1		
	Chien														1				
I V	Rat	1			1													2	
	Souris		1																
	Lapin		1																
Int per	Rat	2		2				2		1				1		1			
I M	Rat	4		10			3	8	2	1		1		1	2		1		
	Souris		1	2				2											
I R	Rat		2	5	2	1	2		1										
S C	Rat	1		1															
I F	Rat	2																	

Abréviations
- Inh = inhalation
- Itr = intratrachéal
- Int nas = intranasal
- Int bro = intrabronchique
- Int plo = intrapleural
- I V = intraveineux
- Int per = intrapéritonéal
- I M = intramusculaire
- I R = intrarénal
- S C = sous cutané
- I F = intrafémoral

IV- OBJECTIVES FOR THE NEXT REPORTING PERIOD

1- Procéder à une analyse statistique des relations entre les indicateurs biologiques à l'aide de bases de données

2- Etendre la comparaison à d'autres métaux non ferreux (cadmium)

V. OTHER RESEARCH GROUP(S) COLLABORATING ACTIVELY ON THIS PROJECT

VI. PUBLICATIONS

- R. Maximilien - Comparaison du détriment radiologique et chimique chez l'homme. Rapport C.E.A. R-5446 1988

- G. Dean, M.A. Anderson, R. Maximilien - Increased risk of cataract in patient receiving radiotherapy to the eye : a pilot study. Brit. J. Radiol. 1988,61,309-311

TITLE OF THE PROJECT N°3 CONTRACT B16-122-F (D)

Evaluation des dimensions subjectives du détriment radiologique en relation avec les considérations sociologiques.

HEAD(S) OF PROJECT
J.BRENOT
I.P.S.N./D.P.S.
BP n° 6
92265 FONTENAY AUX ROSES
Cedex

SCIENTIFIC STAFF

J.BRENOT, S.BASTIDE

I. OBJECTIVES OF THE PROJECT

L'analyse des attitudes du public envers l'énergie nucléaire montre l'importance des composantes subjectives dans l'appréciation du risque. L'expérience, l'anxiété, l'aversion pour le risque ne sont pas les seuls facteurs à prendre en compte ; en effet, l'idéologie et la culture jouent un rôle essentiel dû au fait que toutes les activités nucléaires suscitent l'intérêt quand elles ne provoquent pas des conflits sociaux. La recherche dans une approche comparative conduit à analyser :

* Les dimensions subjectives dans la perception du risque radiologique et des autres risques.
* Les différences de perception qui se manifestent au sein du public et chez les décideurs.

L'objectif final est de proposer des méthodes ou tout au moins des recommandations pour intégrer cette subjectivité dans la gestion du risque.

II. OBJECTIVES OF THE REPORTING PERIOD : 1988

1. Bibliographie
2. Publication et présentation des travaux réalisés dans divers colloques
3. Perception des risques par les experts
4. Suivi de l'impact de Tchernobyl dans l'opinion

III. PROGRESS ACHIEVED

1. Bibliographie

Communiquer sur les risques fait l'objet de nombreux articles ou synthèses qui présentent soit des pratiques, soit des recommandations, ou encore qui traitent de la communication comme une stratégie et qui, de ce fait, soulèvent des problèmes d'éthique.

2. Promotion des travaux réalisés

Quatre textes traduisent l'état d'avancement de la recherche entreprise.

* Le premier (1), présenté à Nice, traite du danger perçu par le grand public pour de nombreuses activités. Les domaines nucléaire et chimique sont comparés au plan des perceptions ; les différences d'opinion entre les individus du public et les travailleurs mettent en évidence le rôle majeur que joue l'expérience du risque.

* Le deuxième (2), présenté à Nice, développe le contexte institutionnel et décisionnel dans lequel se déroulent les études de risque. La sécurité étant un enjeu, les décisions sont souvent issues de situations conflictuelles.

* Le troisième (3), présenté à Stockholm, aborde le problème de communiquer sur le risque. Cette communication diffère selon que l'on tient compte de la personnalité de l'individu, selon que l'on s'adresse à des sujets qui vivent le risque, ou finalement selon que l'on expédie des messages à des individus pour lesquels le risque n'est que représentation.

* Le quatrième (4), présenté à Laxenburg, développe les deux grandes approches de la perception des risques. Dans l'approche mécaniste, la perception d'un risque est fonction des caractéristiques objectives du risque et de la personnalité de l'individu. Dans l'approche constructionniste, la perception dépend non seulement de ce qui vient d'être mentionné mais encore des discours et commentaires faits sur le risque par les médias et les principaux acteurs du domaine. Les aspects techniques et idéologiques interviennent donc dans la représentation ; le risque est une construction sociale et sa perception est soumise à une dynamique.

3. Perception du risque par les experts

Ce sujet n'a pas été avancé comme il était prévu. Seul un test du questionnaire a été réalisé. Le questionnaire demande à être encore repris avant d'être adressé aux experts pour passation.

4. Suivi de l'impact de Tchernobyl dans l'opinion

On a pu constater après l'accident de Tchernobyl des modifications d'opinion sur le nucléaire au sein de certains groupes sociaux. Le groupe des agriculteurs a fait l'objet d'une étude particulière. Un échantillon

représentatif (244 individus) a été enquêté parallèlement à la réalisation d'une enquête nationale en Février 1987. Le document (5) rassemble les résultats. Brièvement, on peut souligner que les agriculteurs ont été plus impressionnés par l'accident que l'individu moyen du public ; qu'ils ont une plus grande inquiétude sur les risques des centrales nucléaires et qu'ils font preuve d'un plus grand scepticisme vis à vis des structures d'information que le Français moyen.

IV- OBJECTIVES FOR THE NEXT REPORTING PERIOD

1. Terminer l'étude sur la perception des risques chez les spécialistes de la sureté et de la protection. Deux questionnaires leur seront remis : l'un détaillé sur les caractéristiques des risques existant dans certaines activités nucléaires, industrielles ou agricoles ; le second reprenant les activités et l'échelle de danger qui avaient déjà été proposées au grand public.

2. Réaliser un ensemble d'entretiens non directifs (100 à 200) au sein du grand public. Les points de l'entretien seront dans l'ordre :

* Préoccupations générales des individus
* Où sont les risques parmi celles-ci ?
* Pourquoi ces risques ont-ils été mentionnés ?
* et plus précisément, comment l'individu gère-t-il ces risques (autonomie ou délégation) ?
* enfin, les activités industrielles et technologiques génératrices de risques seront explicitement abordées.

V. OTHER RESEARCH GROUP(S) COLLABORATING ACTIVELY ON THIS PROJECT

Groupe de Recherche Energie, Technologie et Société (GRETS)
Direction des Etudes et Recherches. Electricité de France
30, rue de Condé, 75006 PARIS.

VI. PUBLICATIONS

1. BASTIDE S., BRENOT J.
Risk perception in the public : results of a survey. In : Séminaire sur les applications, les perspectives et les limitations des évaluations comparatives des risques en vue de leur gestion, Nice, 26-30 Septembre 1988.

2. PAGES J.P , CARDE C., BRENOT J., BASTIDE S.
Analysis of risk perception. Decision context and approaches. In : Séminaire sur les applications, les perspectives et les limitations des évaluations comparatives des risques en vue de leur gestion, Nice, 26-30 Septembre 1988.

3. BRENOT J., PAGES J.P.
Risk, opinions and communication. In : Symposium on management of risk from genotoxic substances in the environment, Stockholm, Oct. 3-6 1988.

4. PAGES JP., BRENOT J., BASTIDE S., CARDE C.
Risk perception, conflicts and decisions. In : First European Conference "Utility of risk analysis in decision making", IIASA, Laxenburg, Nov.1988.

5. BRENOT J., BARNY M.H.
Huit mois après Tchernobyl. Image du nucléaire chez les agriculteurs. Note LSEES 88/17, Juin 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Université Paul Sabatier
Division des Affaires Scientifiques
118, Route de Narbonne
F-31062 Toulouse Cedex

Contract no.: BI6-F-122-F
Sub Contract SC-003-F

Head(s) of research team(s) [name(s) and address(es)]:

Dr. M. Delpoux
Lab. de Botanique et Biogéographie
Université Paul Sabatier
39 Allée Jules Guesde
F-31062 Toulouse Cedex

Telephone number:

Title of the research contract:

Comparative genotoxicity of the principal environmental agents.

List of projects:

1. Comparative genotoxicity of the principal environmental agents.

Title of the project no.: 2

Evaluation du détriment objectif global et de son coût en relation avec les considérations économiques - Recherches sur le végétal

Head(s) of project: M. DALEBROUX

Scientific staff: M. DALEBROUX
M. DELPOUX

I. Objectives of the project:

Etude de la génotoxicité comparée des principaux facteurs chimiques et physiques de l'environnement.

II. Objectives for the reporting period:

- Evaluation de la mutagénicité globale de configurations variées d'environnement : modifications et améliorations à apporter au dispositif expérimental, expériences in situ en milieux urbains, industriels et professionnels.

- Evaluation de la mutagénicité d'atmosphères faiblement enrichies en gaz polluants : modification du dispositif générateur de mélanges gazeux à faibles teneurs en polluants. Répétition de l'expérimentation avec le SO_2 et premiers essais avec NO_x.

- Evaluation de la mutagénicité du radon : expérimentation avec des concentrations inférieures à 1 000 CMA.

- Caractérisation de la relation dose-effet pour de faibles doses d'irradiation du dispositif d'irradiation réalisés au moyen d'une source de cobalt 60 : modification du dispositif et étude d'un gradient plus proche du point zéro.

lll. Progress achieved :

L'année a été essentiellement consacrée à des travaux techniques destinés à modifier ou réaliser des dispositifs adaptés aux objectifs biologiques.

1/ Modification du dispositif d'évaluation in situ de l'impact global de l'environnement:mise en placed'une réserve en eau permettant d'assurer une humidité relative égale à 100% dans les dispositifs de culture.

2/ Construction d'un dispositif d'inhalation du radon adapté à la culture du Tabac : la première expérience relatée dans un précédent rapport avait été réalisée dans un dispositif d'inhalation pré-existant développé au Laboratoire de Pathologie pulmonaire de la COGEMA à Razès (Haute-Vienne, France) pour faire inhaler du radon à des petits mammifères. Ce dispositif ne comportait en particulier pas de système d'éclairage. Pour assurer le développement des plants de Tabac, un système provisoire avait été construit. Sur la base des résultats positifs obtenus dans l'expérience préliminaire, il a été conçu et réalisé au cours de l'année 1988 un dispositif d'inhalation nouveau et indépendant des structures pré-existantes. Outre trois caissons étanches branchés sur les conduites de distribution du radon, des dispositifs lumineux fixes ont été disposés pour faire croître les plants de Tabac dans des conditions optimales.

3/ Modification du dispositif d'irradiation au Cobalt 60 de la Section de Toxicologie et Cancérologie expérimentale de l'IPSN-DPS-SPE, CEA, Bruyères le Chatel, Essonne, France : Les premiers résultats positifs obtenus avec des rayonnements émis par la source au cobalt 60 désigné ci-dessus nous ont conduit à proposer des expérimentations mettant en jeu des irradiations caractérisées par des débits 10, 100 et 1000 fois moins élevés que ceux mis en oeuvre dans la première expérience. Pour cela, il a été nécessaire de demander la fabrication de trois nouvelles sources et de les placer par activité décroissante dans la chambre d'irradiation de Bruyères le Chatel.
Des difficultés administratives puis techniques (nécessité de fabriquer deux fois les sources) ont retardé les opérations mais tout devrait être en mesure de fonctionner à partir du début du mois de février 1989.

Remarques :

- les engagements financiers liés à cette dernière opération nous ont conduit à différer le développement du dispositif destiné à remplacer le prototype utilisé pour étudier au laboratoire les effets génétiques du SO_2. Ce développement sera effectué pendant l'année 1989.

- parallèlement aux développements technologiques évoqués ci-dessus, une mise au point scientifique a été réalisée sous la forme de thèses présentées devant l'Université Paul Sabatier par trois étudiants (Pierre MAGNES, Sophie MURATET et Odile VERNET) : soutenances les 6 et 26 janvier 1989. Ces thèses ont été en partie rédigées avec des données obtenues dans le cadre du programme donnant lieu à ce rapport.

IV. Objectives for the next reporting period

- étude de la forme de la réponse dose-effet génétique autour des sources de cobalt 60 qui seront successivement installées à la Section de Toxicologie et Cancérologie expérimentale de l'IPSN-DPS-SPE, CEA, Bruyères le Chatel.

- étude des effets génétiques du radon avec des atmosphères diversement enrichies en radon grâce au dispositif construit à la COGEMA de Razès (Haute-Vienne, France)

- approfondissement de l'étude des effets génétiques du SO_2.

- évaluation in situ des effets génétiques d'atmosphères diversement polluées.

V. Other research group(s) collaborating actively on this project (name(s) and adress(es))

- Laboratoire de Pathologie Pulmonaire Expérimentale de la COGEMA, Razès, Haute-Vienne, France (Dr CHAMEAUD)

- Section de Toxicologie et Cancérologie Expérimentale de l'IPSN-DPS-SPE, CEA, Bruyères le Chatel, Essonne, France (Dr METIVIER)

- Laboratoire de Chimie-Energie-Environnement de l'Ecole Nationale de Chimie de l'Institut National Polytechnique de Toulouse, 118 Route de Narbonne, Toulouse, France (Dr TORRES, Professeur)

- Laboratoire de Mutagénèse de la Station d'Amélioration des Plantes de l'INRA, F-21034, Dijon Cedex (Dr DULIEU)

- Union Technique de l'Automobile du Motocycle et du Cycle, Autodrome de Linas-Montléry (M. ING, Mme B. LOPEZ)

VI. Publications

DELPOUX M. et M.A. DALEBROUX - Effets génétiques de sols du Pays de Sault (Aude) différant par leur radioactivité naturelle et leur composition chimique,sous presse in : Le Pays de Sault : Espaces, Peuplements, Populations, Ed. du C.N.R.S., 6 p.

MAGNES P. - Etude des effets génétiques des champs électriques à 50 hertz et d'autres rayonnements d'origine naturelle et artificielle, Thèse Université Paul Sabatier de Toulouse, 1989, 178 p.

MAGNES P., M. DELPOUX et M.A. DALEBROUX - Premières investigations pour établir une courbe de réponse étalon du système a1+/a1 a2+/a2 du Tabac à différentes doses de rayonnement gamma du ^{60}Co en vue d'y rattacher les effets génétiques des principaux facteurs de l'environnement. A paraître in Proc. Seminar on Applications, Perspectives and Limitations of comparative Risk Assessment and Risk Managment. CEC, Nice, France, 26-30 september 1988, 15 p.

MURATET S. and O. VERNET - Etude et comparaison des effets génétiques et biologiques de la pollution atmosphérique et de très faibles doses de radioactivité naturelle. Thèse Université Paul Sabatier de Toulouse, 1989, 1 fasc. texte 148 p., 1 fasc. illustr. 191 p.

MURATET S., O. VERNET, M. DELPOUX et M.A. DALEBROUX - Evaluation en laboratoire des effets génétiques du SO_2 sur le système a1+/a1 a2+/a2 de Nicotiana tabacum L. var. xanthi, 8 p. dactyl. (soumis pour publication).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-123-D

Deutsches Krebsforschungszentrum
Institut für Radiologie
und Pathophysiologie
Im Neuenheimer Feld 280
D-6900 Heidelberg 1

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. G. van Kaick
Inst. Radiologie & Pathophysiologie
Deutsches Krebsforschungszentrum
P.O. Box 101949
D-6900 Heidelberg 1

Telephone number: (6221) 48.45.63

Title of the research contract:

Thorotrast - investigations to evaluate the long term effects caused by artificial radiation in man (Thorotrast patients) - follow-up study.

List of projects:

1. Thorotrast - investigations to evaluate the long term effects caused by artificial radiation in man (Thorotrast patients) - follow-up study.

Title of the project no: BI6-F-123-D

Thorotrast - investigations to evaluate the long term effects caused by artificial radiation in man (Thorotrast patients) - Thorotrast follow-up study.

Head(s) of project:

Prof.Dr. G. van Kaick, coordinator of the study
Prof.Dr.Dr.h.c. H. zur Hausen, chairman and scientific member of the German Cancer Research Center,
Prof.Dr. W.J. Lorenz, director of the Institute of Radiology and Pathophysiology (former: Institute of Nuclear Medicine)

Scientific staff:

Dr. H. Lührs, Dr. G. Layer

Statistical evaluation:

Dr. H. Wesch.

I. Objectives of the project:

The aim of the German Thorotrast study is to discover the late effects of incorporated colloidal thoriumdioxide by epidemiological observation and clinical and biophysical examination of the patients; to compare the results of those of a corresponding control group and to assess the relationship between late effects and radiation dose. Furthermore we try to offer appropriate diagnostic and if possible therapeutic facilities and to give advise to the family physicians of the patients.

II. Objectives for the reporting period:

Research activities:
- Clinical, biochemical and radiological examinations of the Thorotrast patients and the control group followed by a report to the family physician
- Biophysical examinations to calculate the tissue dose due to the Thoriumdioxide deposits and their radioactive daughter products
- Identification of the causes of death of Thorotrast patients and members of the control group
- Statistical evaluation of the epidemiological, clinical and biophysical data.

III. Progress achieved:

Clinical results:
In 1988 we performed 150 outpatient examinations (61 of the control group) applying medical, radiological and biophysical methods. In the group of examined Thorotrast patients we uncovered 3 primary malignant liver tumors. Two patients underwent segmental liver resection; one patient with an advanced stage of liver tumor was treated by tumor embolisation. By imaging methods we found recurrence of a liver tumor in one case and distant metastases in another one. Renal cancer was seen at CT-pictures and could be proven by nephrectomy. We firstly diagnosed a chronic lymphatic leukemia in a Thorotrast patient. In 5 patients CT showed lesions in the Thorotrast spleen. Three patients with paravascular Thorotrast deposits suffered from newly developed outer fistula. Diabetes mellitus was equally distributed in Thorotrast and control patients.
Epidemiological results:
Statistical evaluation of the data of deceased patients was performed. We present here only the most important results. Four cohorts were formed to study the dependence of the age at injection on the frequency of liver tumors (1-14; 15-29; 30-44; 45-59 years). The cumulative rate of liver tumors shows the same pattern for all cohorts (Figure 1). A marked increase in the liver tumor rate occurs 30 to 40 years after injection. The curve of the oldest cohort can be seen only in the beginning, as these patients cannot reach the exposure time and the accumulative dose respectively, which are necessary for the induction of the liver tumors.
Figure 2 shows the risk estimate for liver cancer. The calculation was done with the following assumptions: a) Patients who have died within the first 15 years of exposure were excluded from the evaluation as they are not at risk for liver tumors. b) The dose delivered during the last 10 years before clinical manifestation of the tumor will be looked upon as wasted dose, because the tumor already exists growing from microscopical to clinical dimensions! The calculation was done for each patient separately! The total risk was calculated for each year by taking the cumulative number of liver tumors up to that year as numerator and the cumulative dose of all patients as denominator. Those figures were plotted over the years after injection. There is a clear difference between the risk for male and female patients, which comes up after 40 years to 500 and 300 liver tumors per 10^6 person cGy respectively.
During the past years there is a constant trend that Thorotrast patients die earlier compared to patients of the control group. This phenomenon is depending on the amount of Thorotrast injected. However, as the frequency of liver tumors increases with the incorporated volume of Thorotrast, this result could be caused by the high numbers of liver cancer.
Excluding in the analysis those patients who have died by Thorotrast specific diseases (Liver cancer or leukemias or liver cirrhosis) we have similar results in dose rate dependent life-shortening (Figure 3).

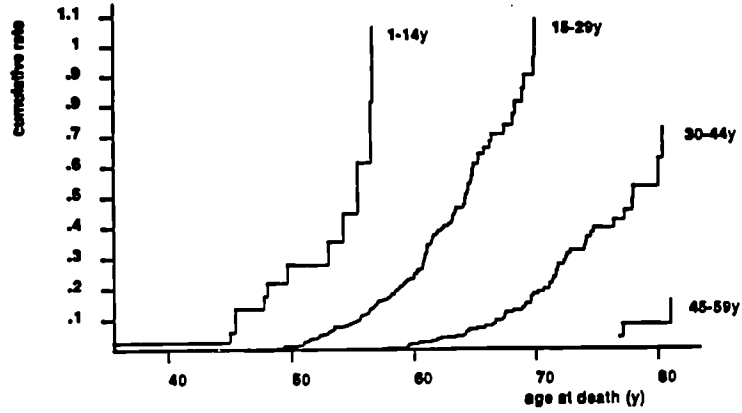


Figure 1
Cumulative rate of liver tumors in Thorotrast patients of the examined group. Four cohorts were formed according to the age at injection:
a) 1-14 years b) 15-29 years c) 30-44 years d) 45-59 years

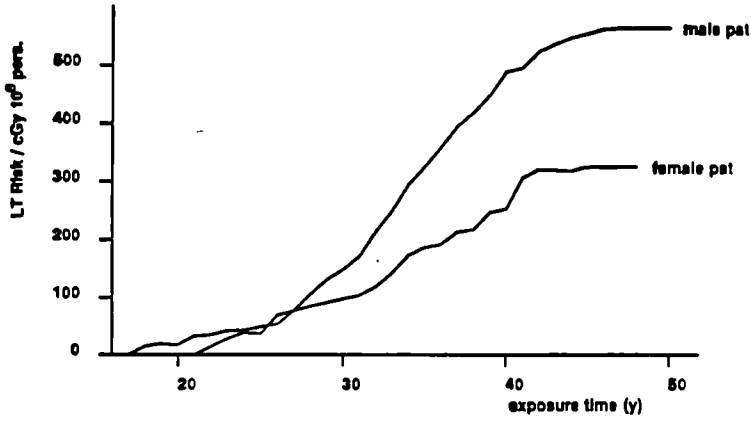


Figure 2
Risk estimates for liver cancer correlated to the years of exposure separated in male and female patients.
Patients of the examined group were combined with patients of the non examined group whose volumes of injected Thorotrast were documented (calculation see text).

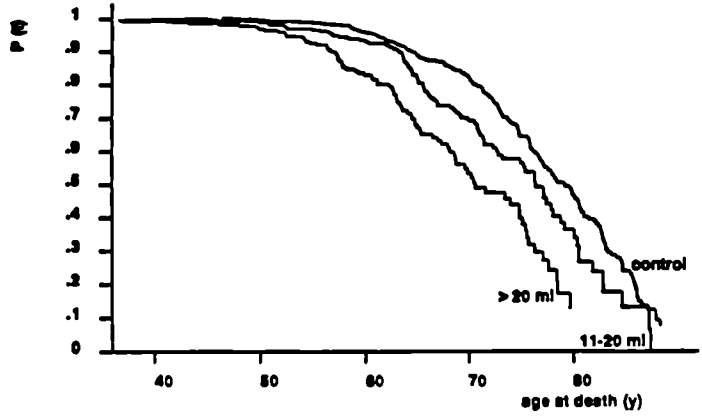


Figure 3
Kaplan-Meier survival curves of the examined patients excluding Thorotrast specific diseases. Three cohorts were formed according to the volume of intravascular injected Thorotrast: a) 1-10 ml b) 11-20 ml c) >20 ml. The curve for the 1-10 ml cohort is omitted to keep the graph readable.

IV. Objectives for the next reporting period:
The working program will be continued according to the recommendations of the coordinating committee

- regular correspondence with about 750 patients of the Thorotrast and control group as well as with the respective family doctors
- computer controlled requests for follow up examinations
- out-patient reexaminations of Thorotrast carriers and patients belong to the control group at two-year intervals
- use of diagnostic ultrasound, computerized tomography, nuclear medical and immunological diagnostic methods for the uncovery of Thorotrast induced neoplastic diseases
- biophysical examinations of Thorotrast carriers (measurements with the whole body counter and exhalation measurements)
- computer suitable registration of examination data and interception of comprehensive medical reports for the family doctors as well as the treating hospitals
- supplementation of the time orientated information system for statistical analysis of the examination results for the Thorotrast and control patients
- controlling of the stored data and preparation of final statistical evaluation

V. Other research group(s) collaborating actively on this project (name(s) and address(es)):

Prof.Dr. H. Muth (em.). Inst. f. Biophysik, Univ.d.Saarlandes, D-6650 Homburg/Saar
Prof.Dr. A. Kaul, Bundesgesundheitsamt, Ingolstädter Landstr. 1, D-8042 Neuherberg
Prof.Dr. K. Wegener, Städt.Krankenanstalten, Path.Inst., Bremserstr. 79, D-6700 Ludwigshafen
Prof.Dr. G. Wagner (em.), Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg
Prof.Dr. H. Immich (em.), Inst.f.Med.Dok.u.Statistik. d.Univ., Im Neuenheimer Feld 325, D-6900 Heidelberg
Prof.Dr. Ch.Mays, Department of Health and Human Services, Rad. Epidemiology Br, National Cancer Institute, National Institutes of Health, Building EPN Room 408, Bethesda/Maryland 20892, U.S.A.

VI. Publications:

Dalheimer, A.R.; A. Kaul:
Calculation of local dose to tissues adjacent to Thorotrast aggregates.
Presentation at the Workshop "Risks from Radium and Thorotrast", October 3-5, 1988, Bethesda U.S.A.

Hornik, S.; A. Kaul:
Calculation of the basal cell dose in Thorotrast patients.
Presentation at the Workshop "Risks from Radium and Thorotrast", October 3-5, 1988, Bethesda U.S.A.

Kaick, G. van; H. Wesch; H. Lührs; D. Liebermann; A. Kaul; H. Muth:
The German Thorotrast study - report on 20 years follow-up.
Presentation at the Workshop "Risks from Radium and Thorotrast", October 3-5, 1988, Bethesda U.S.A.

Muth, H.:
History of the German Thorotrast studies.
Presentation at the Workshop "Risks from Radium and Thorotrast", October 3-5, 1988, Bethesda U.S.A.

Spiethoff, A.; H. Wesch; K. Wegener:
Tumor induction in rat liver by fractioned irradiation with neutrons and foreign body burden in comparison to Thorotrast induced tumors.
Presentation at the Workshop "Risks from Radium and Thorotrast", October 3-5, 1988, Bethesda U.S.A.

Wegener, K.; H. Wesch; K. Küttler; A. Spiethoff:
Thorotrastosis in humans and animals. Pathoanatomical results of the German Thorotrast study.
Presentation at the Workshop "Risks from Radium and Thorotrast", October 3-5, 1988, Bethesda U.S.A.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

International Commission on
Radiological Protection (ICRP)
P.O. Box 35, Didcot
GB- Oxon OX11 ORJ

Contract no.: BI6-F-124-UK

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H. Smith
ICRP
P.O. Box 35, Didcot
GB- Oxon OX11 ORJ

Telephone number: (0235) 833929

Title of the research contract:

Development of fundamental data for radiation protection.

List of projects:

1. Development of fundamental data for radiation protection.

Title of the project no.: 1

Head(s) of project:
Dr H Smith

Scientific staff:

I. Objectives of the project:

Evaluating the biological basis of radiation-induced effects and the metabolism and dosimetry of incorporated radionuclides are integral parts of the basic recommendations on radiation protection. In this respect, the Main Commission requires an input from its Committees, aided by task groups, who critically evaluate all available data at the request of the Main Commission. Reports prepared by committees are considered by the Main Commission and, if adopted, are published by the Secretariat. The head of the project plays a coordinating role in this process.

II. Objectives for the reporting period:

To review and adopt reports already prepared for the Main Commission, to continue with the development of additional fundamental data and to identify and discuss topics relevant to the revision of the ICRP basic recommendations.

III. Progress achieved:

1. Introduction

The ICRP continues to maintain close liaison with several international organisations by means of attending relevant meetings and providing advice when requested to do so. The Main Commission, its Committees and Task Groups met as detailed below during 1988.

Group	Meeting place
Main Commission	Bariloche, Argentina
Committee 1	Bath, England
Committee 2	Seattle, USA
Committee 3	Budapest, Hungary
Committee 4	Chilton, England
Task Groups:	
Revising the basic recommendations	Bethesda, USA Long Island, NY, USA
Risk estimates for cancer and genetic effects	Bath, England
Biological basis for dose limitation to the skin	Bethesda, USA
RBE for nonstochastic effects	Rijswijk, Netherlands
Revision of Reference Man (Publication 23)	Paris, France
Age dependent dosimetry	Oak Ridge, USA
Radiological protection of workers in medicine and dentistry	Budapest, Hungary
Revision of ICRP Publication 40	Paris, France
Optimisation and decision making in radiological protection	Chilton, England
Probabilistic exposures	Vienna, Austria

2. Progress reports

2.1 Main Commission

2.1.1 Introduction

A major discussion took place at Bariloche regarding the concepts to be used in new basic recommendations in radiological protection. As a result, a progress report was issued.

Two reports were adopted. These were:-

- Optimisation and decision making in radiological protection. (Subject to a postal ballot to approve minor changes in the text.)
- Principles for the protection of the patient in diagnostic radiology (to be published as a note in the Annals, and in addition, circulated to members of the International Congress on Radiology, Paris 1989).

2.2 Main Commission Task Group

2.2.1 Revision of the basic recommendations

The new recommendations will take account of new biological information and of current developments in radiological protection. In this respect the Task Group is relying upon input from several other task groups.

The report will contain a main text supported by more detailed annexes. In addition to retaining the general form

of the system of dose limitation, the possibility is being considered of supplementing the dose limit with selectively applied, source-related dose constraints.

Natural sources and sources where control cannot be applied at source will also be discussed separately to situations where control of the source is possible.

The magnitude of the limits to be recommended will have to await advice from Committee 1's Task Group on Risk Estimates for Cancer and Genetic Effects.

It is hoped that an advanced draft of the report will be reviewed by the Main Commission in April 1989 and to deliver a complete draft to the 1989-93 Commission at its first meeting in October 1989.

2.3 Committee 1 Task/Working Groups

2.3.1 Risk Estimates for Cancer and Genetic Effects

Estimates of cancer risk appropriate for radiation protection purposes are being prepared for the major organs of the body. Where possible, sex and age dependence of these risks is being taken into account.

Estimates of genetic risk will be based upon data published by UNSCEAR and in the forthcoming BEIR V report, in which multifactorial disorders are excluded.

Consideration is also being given to teratogenic effects, in particular, the dose response relationship for severe mental retardation as a function of time of irradiation during pregnancy.

A report is to be prepared for mid-1989, for use by the Task Group revising the basic recommendations and for final review by Committee 1 in October 1989.

2.3.2 Biological basis for dose limitation to the skin

Decisions are being made as to whether excess cancer or nonstochastic effects are the major concern in irradiated skin and the depth at which dose should be measured. The choice of a weighting factor for skin depends upon these decisions.

A final draft is planned for review by Committee 1 by March 1989 for submission to the Main Commission by July 1989.

2.3.3 RBE for nonstochastic effects

Two situations are being considered - accidental exposure to inhaled alpha and beta-emitting radionuclides; and chronic exposure of all tissues to external radiation at an effective dose equivalent in the tens of mSv range. It should be possible from these data to judge the adequacy of Q factors for establishing ALI's that are determined by nonstochastic effects. A final draft is planned for review by Committee 1 by May 1989 for submission to the Main Commission by July 1989.

2.3.4 Satisfactory progress has been made in working groups associated with hereditary effects, hormesis risks to brain and bladder, reference radiations (x-rays or gamma rays), eye (cataracts), the ongoing review of epidemiology and the possible use of experimental animal data in predicting radiation-induced cancer risk.

2.4 Committee 2 Task/Working Groups

2.4.1 Respiratory Tract Models

A simple and practical model incorporating retention and clearance parameters and dose to tissues at risk is in an advanced stage of preparation. The model will consider mechanical clearance independently of the solubility of inhaled particles, resulting in time varying mechanical and solubility functions. It is still to be decided whether or not to include pulmonary lymph nodes as part of the pulmonary tissue when estimating dose. A final draft report is planned for submission to Committee 2 by October 1989.

2.4.2 Revision of ICRP Publication 23 (Reference Man)

Several of the proposed twenty four chapters covering anatomical and physiological parameters related to age, sex and regional variations are well advanced. A first draft of those completed chapters should be available by mid 1989. A complete draft is anticipated for review by Committee 2 in October 1989.

2.4.3 Age dependent dosimetry and dose per unit intake for members of the public

Evaluation of dose on a first category of radionuclides following their ingestion is nearing completion and a decision is to be made whether or not to await information on the new respiratory tract model before completing dose evaluation following inhalation. A draft is to be submitted to an editorial group in January 1989 and thereafter to the Main Commission at their April 1989 meeting.

2.4.4 Satisfactory progress has been made on working groups concerned with the development of anthropomorphic phantoms for use in the revision of Reference Man; and on the metabolism of tritium and carbon 14 compounds. A report on the latter topic will be published in the open literature.

2.5 Committee 3 Task Groups

2.5.1 Protection of the Worker in Medicine and Dentistry

A draft of this report is in the hands of an editorial group and it is anticipated that a final draft will be submitted to the Main Commission for adoption in April 1989. The report is directed towards the managing authority in hospitals as well as to individual workers in medical and dental practices. The report is also intended for relevant statutory authorities that are responsible for the enforcement of safety standards and for establishing training standards for workers; and to those involved in the planning of medical and associated technical services.

2.6 Committee 4 Task Groups

2.6.1 Revision of ICRP Publication 40 (Protection of the public in the event of major radiation accidents: principles for planning)

This report will provide a logical set of principles to cover both the public and workers. It will include the impact at longer times and greater distances from the accident; and it will indicate upper and lower bounds for a variety of

intervention levels and countermeasures, based upon the principles of optimisation and risk-benefit analysis. A draft report will be considered by Committee 4 at its meeting in March 1989 and the information used by the Task Group revising the basic recommendations.

2.6.2 Probabilistic exposures

This report will provide information to the Task Group revising the basic recommendations on how to apply the principles of radiation protection and safety in considering exposures not intended to occur. It will include both workers and members of the public in this context.

A draft report will be submitted to Committee 4 at its meeting in March 1989.

IV. Objectives for the next reporting period:

To continue the work in progress as defined in Section III. Future meetings are planned by the Main Commission (Paris) the Main Commission and its Committees (Oxford), Committee 4 (Washington) the Task Groups revising the basic recommendations (Oxford, Stockholm), Age dependent dosimetry (Munich), RBE for non-stochastic effects (San Francisco). Meetings are expected to be held by the Task Groups on Skin Risk Estimates, Reference Man, Respiratory Tract Models, and Revision of ICRP Publication 40.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Nil

VI. Publications:

The following reports were published in the Annals in 1988.

ICRP Publication 51.	Data for Use in Protection against External Radiation
ICRP Publication 52.	Protection of the Patient in Nuclear Medicine
ICRP Publication 53.	Radiation Dose to Patients from Radiopharmaceuticals
ICRP Publication 54.	Individual Monitoring for Intakes of Radionuclides by Workers: Design and Interpretation

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-138-NL

Radiobiological Institute
TNO
Division of Health Research
151 Lange Kleiweg
NL-2280 HV Rijswijk

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.J. Broerse
TNO
Radiobiological Institute
151 Lange Kleiweg
NL-2280 HV Rijswijk

Dr. J. Zoetelief
TNO
Radiobiological Institute
151 Lange Kleiweg
NL-2280 HV Rijswijk

Telephone number: 015/13.69.40

Title of the research contract:

Absorbed dose assessments in diagnostic radiology, nuclear medicine and radiotherapy with respect to the female breast.

List of projects:

1. Absorbed dose assessments in diagnostic radiology, nuclear medicine and radiotherapy with respect to the female breast.

Title of the project no.:

Assessment of absorbed dose in the female breast in diagnostic radiology

Head(s) of project: Prof.dr. J.J. Broerse and Dr. J. Zoetelief

Scientific staff: Prof.dr. J.J. Broerse, N.J.P. de Wit and Dr. J. Zoetelief

I. Objectives of the project:

Because of the high incidence of mammary cancer in the member states of the European Communities, screening projects employing mammography are carried out. In the Netherlands (and the UK), nation wide population screening is planned to be implemented in 1988. Therefore, it is essential that absorbed doses in mammography can be determined in relation to the image quality of the mammograms. Such studies will be required to formulate protocols for quality assurance as well as to provide recommendations for dose reduction in mass-screening programs. Absorbed dose values in the breast have to be derived from measurements in phantoms combined with information on the anatomical structure and size of the breasts. Data obtained from actual screening programs will lead to the assessment of the contribution of mammography to the total radiation burden of the population.

II. Objectives for the reporting period:

A protocol for quality control was tested at the Comprehensive Cancer Centre Rotterdam. Limiting values were derived for variations in film processing, total mammography procedure and tube voltage. Deviations in compressed breast tissue composition from the average were investigated by estimations of percentages of glandular tissue and mAs measurements for mammograms of compressed breasts. Investigations of film processing and doses required for new types of film/screen combinations were continued. An analysis was performed of screen/film mammography in terms of information theory.

III. Progress achieved:

To maintain an optimum in the risk/benefit balance, a quality control program concerning the technical and dosimetrical aspects of mammography has to be implemented. A quality control protocol was drafted, which includes constancy checks at different time intervals:

- For each woman the compressed breast thickness and focal spot charge (mAs) are registered;
- Daily checks of the film processing are carried out using sensitometry/-densitometry;
- Daily a radiograph is made of a reference phantom (for which the focal charge is recorded and the average density is determined);
- Approximately three-monthly, absorbed dose measurements are made with an ionization chamber inside a polymethylmethacrylate (pmma) phantom;
- With reference to the automatic exposure control unit (AEC) the radiation quality is assessed and the focal spot size is determined.

The quality control protocol was implemented and tested at the mammography unit of the Comprehensive Cancer Centre Rotterdam (BOC-IKR).

Film processing

3M-MS films are exposed by an X-rite type 333 sensitometer (step size 0.15 D) and processed with a 3 M type 515 processor. In Table I, for sensitometer step number 10, the average density ($d_{\bar{}}$) and standard deviation as measured with a MacBeth transition densitometer type TD931 are shown for various periods. The standard deviation for the last three periods is about 4 per cent. In the nine-month period, the value of $d_{\bar{}}$ decreases by about 25 per cent, which is significant. The curves of photographic density as a function of sensitometer step number kept the same steepness and showed in course of time a shift to higher step numbers. This suggests that the density of sensitometer step 10 provides a reasonable indication of the constancy of film processing. Concerning quality control (constancy) it is concluded that a deviation of 15 per cent from the average value should result in a check of the processing procedure (i.e. film batch, temperature, process time, replenishment).

TABLE 1

RESULTS OF SENSITOMETRY/DENSITOMETRY IN COURSE OF TIME
(mean and standard deviation of density at step 10)

period	$d_{\bar{}}$ (density at step 10)
870310 - 0403	1.236 (1 ± 0.040)
870406 - 0429	1.161 (1 ± 0.070)
870429 - 0521	1.129 (1 ± 0.051)
870521 - 0612	1.159 (1 ± 0.067)
870615 - 0710	1.068 (1 ± 0.062)
870713 - 0822	1.058 (1 ± 0.063)
870824 - 0909	1.086 (1 ± 0.051)
870923 - 1015	1.014 (1 ± 0.038)
871016 - 1113	1.009 (1 ± 0.035)
871116 - 1211	0.964 (1 ± 0.038)

Radiographs of reference phantom

Daily radiographs of the reference phantom (Wisconsin Mammographic Random Phantom (RMI, USA)) provide information about the total mammography procedure. For each radiograph the focal spot charge (fc) value and the average density ($\bar{d}$) are determined. In addition, the phantom can be used to assess the physical image quality. In Table II the mean values and standard deviations of $\bar{d}$, fc and d_s are presented for a nominal tube voltage of 30.5 kV, less density (lower level of the AEC) and a grid. Also given in this table are the mean ratios of $\bar{d}/d_s$, $\bar{d}$/fc and $\bar{d}/(d_s.fc)$ and the standard deviations. The standard deviations in $\bar{d}$ for different time periods vary from about 3 to 10 per cent, a typical value being about 7 per cent. With respect to constancy, it is concluded that values deviating by more than 20 per cent from the mean value should result in a check of the procedures.
The focal spot charge (mAs) values show standard deviations of about 5 to 16 per cent, a typical value being about 8 per cent.
The image quality assessed in presence of the grid (4.0) is higher than in absence of the grid (3.3), but is resulting in an increased absorbed dose (factor of about 2.4).

TABLE II

EVALUATION OF QUALITY CONTROL MEASUREMENTS AND REFERENCE DOSIMETRY

Tube voltage: 30.5 kV*; less density; grid; image quality: 4.0 ± 0.5

data	$\bar{d}$	fc (mAs)	d_s	$\bar{d}/d_s$	$\bar{d}$/(fc) (C^{-1})	$\bar{d}/(d_s.fc)$ (C^{-1})	D_{en} (mGy)	$\bar{D}$ (mGy)
870521	0.963	31.9	1.16	0.831	31.0	26.3		
870612	(0.081)	(0.041)	(0.067)	(0.039)	(0.070)	(0.038)		
870619		29.4					3.98	1.06
870615	0.829	28.0	1.07	0.772	29.5	27.7		
870710	(0.080)	(0.080)	(0.062)	(0.044)	(0.058)	(0.067)		
870713	0.835	28.5	1.06	0.790	29.3	27.7		
870822	(0.064)	(0.073)	(0.063)	(0.055)	(0.063)	(0.052)		
870807		34.7					4.49	1.20
870824	0.895	29.2	1.09	0.825	30.8	28.4		
870909	(0.028)	(0.045)	(0.051)	(0.035)	(0.038)	(0.059)		

* nominal tube voltage
$\bar{d}$ = average density of radiograph of Wisconsin phantom, fc = focal charge, d_s = density of sensitometer step 10, d_{en} = entrance dose at 49 mm thick pmma phantom, $\bar{D}$ = average dose in 49 mm thick pmma phantom, () = measure standard deviation.

Absorbed dose measurements with reference to the AEC unit

The entrance and average absorbed dose values for a 49 mm thick pmma phantom given in Table II (together with the mAs values) show considerable differences (normal variations are ± 4 per cent). The dosimetric measurements suggest instabilities in the tube voltage in course of time. For a 50 mm thick phantom and 0.5 kV difference in tube voltage a change with a factor of about 1.13 for the entrance dose at constant exit dose is found. A difference of 1 kV in tube voltage results in differences in entrance dose of about 28 per cent. This suggests a limiting value of ± 0.5 kV for variations in tube voltage.

IV. Objectives for the next reporting period:

The quality assurance protocol will continously be tested at the Comprehensive Cancer Centre Rotterdam with emphasis on different mammography units. Investigations of film/screen combinations in dependence on film processing will be performed in terms of absorbed dose and image quality. In addition, characteristics of AEC units will be studied.
The investigations of the influence of compressed breast tissue composition on absorbed dose in relation to the mAs values will be continued for actual mammograms.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Dr. J.J. Paulides. Comprehensive Cancer Centre Rotterdam, Rotterdam, The Netherlands.
- Dr. A.H.L. Aalbers. National Institute for Public Health and Environmental Hygiene, Bilthoven, The Netherlands.
- Dr. H.W. Julius, Radiological Service TNO, Arnhem, The Netherlands.
- Dr. F. van der Meer. Department of Radiology. Medical Faculty of the University of Rotterdam, Rotterdam, The Netherlands.

VI. Publications:

- J. Zoetelief. Mammografie. In: Grondbeginselen stralingsfysica en radiobiologie voor medische toepassingen (Eds. J.J. Broerse, L.A. Hennen, A.F. Hermens en J. Zoetelief). IRS-TNO, 1987.
- J. Zoetelief, N.J.P. de Wit and J.J. Broerse. Technical and dosimetrical aspects of quality control in mammography (CEC-workshop Brussels, February 1988). In press.
- J. Zoetelief, N.J.P. de Wit and J.J. Broerse. Technique for monitoring absorbed dose to the breast in mammography (NRPB-CEC workshop, September 1988, Oxford). In press.
- W.A. Hummel: Systeemanalytische beschrijving van het beelvormende systeem voor mammografie. Afstudeerverslag, TU Delft, January 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-139-I

Università degli Studi di Pisa
Lungarno Pacinotti 43/44
I-56100 - Pisa

Head(s) of research team(s) [name(s) and address(es)]:

Prof. L. Donato
Istituto di Patologia Spec. Med.
dell'Università di Pisa
Via Roma 67
I-56100 - Pisa

Telephone number: 050/47231

Title of the research contract:

Limitation of patient exposure to radiation from emerging medical diagnostic procedures in high morbidity disease areas.

List of projects:

1. Nuclear cardiology versus two-dimensional echocardiography for detection, quantitation and follow-up studies of myocardial disease.
2. Diagnostic efficacy of immunoscintigraphy with radioactive monoclonal antibodies (versus bidimensional echography).

Title of the project no.: 1

Nuclear cardiology versus two-dimensional echocardiography for detection, quantitation and follow-up studies of myocardial disease.

Head(s) of project:

C. Contini, Associate Professor of Clinical Physiology, University of Pisa.

Scientific staff:

D. Neglia, M.D., A. Distante, M.D., O. Parodi, M.D., D. Levorato, M.D., C. Arlotta, M.D., P. Marzullo, M.D., E. Picano, M.D.

I. Objectives of the project:

The project is aimed at the investigation of radiation exposure to patients and operators resulting from nuclear cardiac blood pool gating and other nuclear cardiology procedures largely used to screen suspects of cardiomyopathies (primary or secondary). Moreover the project will attempt to evaluate the role of ultrasounds as an alternative procedure.

II. Objectives for the reporting period:

In patients selected on the suspicion of early cardiomiopathy, because of apparently primitive ventricular arrhythmias and documentation of subclinical ventricular dysfunction by means of 2D-Echocardiography (2D-Echo) and Radionuclide Angiography (RNA), we tested the diagnostic reliability of these techniques as compared to the angiographic, hemodynamic and bioptic evaluation. Results obtained in the arrhythmic patients were also compared with those of a control population of normal subjects.

Possible evolution of the early myocardial disease was assessed by a short term preliminary follow-up consisting of clinical and 2D-Echo evaluation every 6 months.

III. Progress achieved:

Among over 400 subjects referred to our Institute because of ventricular arrhythmias, 27 subjects with complex ventricular arrhythmias, documented at 24-hour Holter recording, and without other clinical evidence of heart or systemic disease were selected.

Regional wall motion abnormalities were documented by non-invasive evaluation in 24/27 cases (89%) involving the right, the left or both ventricles. The agreement between 2D-Echo and RNA in the diagnosis or the exclusion of ventricular dysfunction significantly exceeded chance occurrence for both ventricles ($p<0.01$) (figure 1).

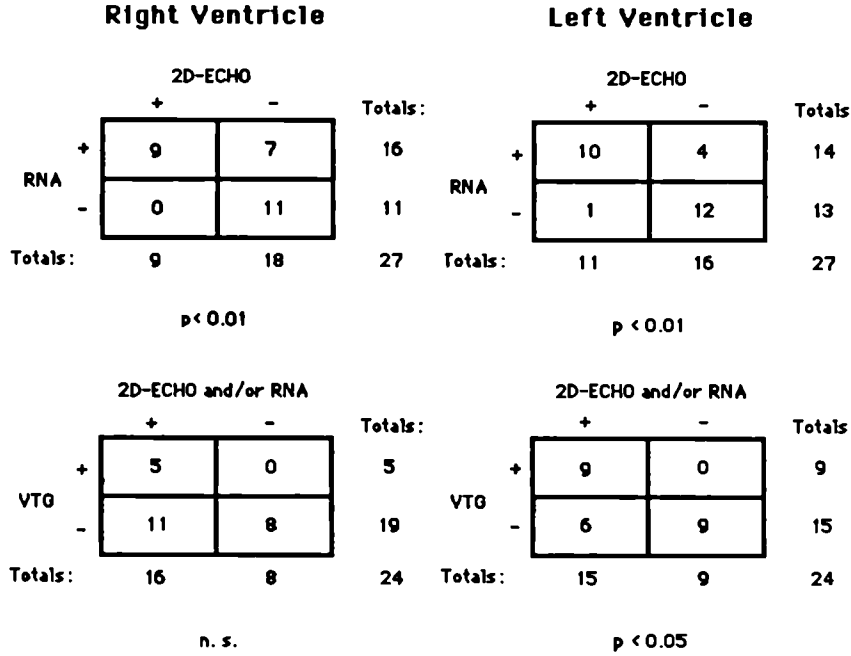


Figure 1: Two by two contingency tables illustrate the concordance between 2D-Echocardiography (2D-ECHO) and RNA and between these 2 combined techniques and contrast ventriculography (VTG) in the diagnosis of right ventricular or left ventricular dysfunction by wall motion analysis (WMI ≥ 2).

Global systolic dysfunction, as assessed by biventricular ejection fraction (EF) values from RNA, was demonstrated in 16/27 patients (59%). Moreover mean biventricular EF values were significantly lower in arrhythmic patients as compared to a control population of 20 normal subjects ($p<0.01$) (figure 2). The extent of EF decrease was also strictly related with the extent of regional wall motion abnormalities.

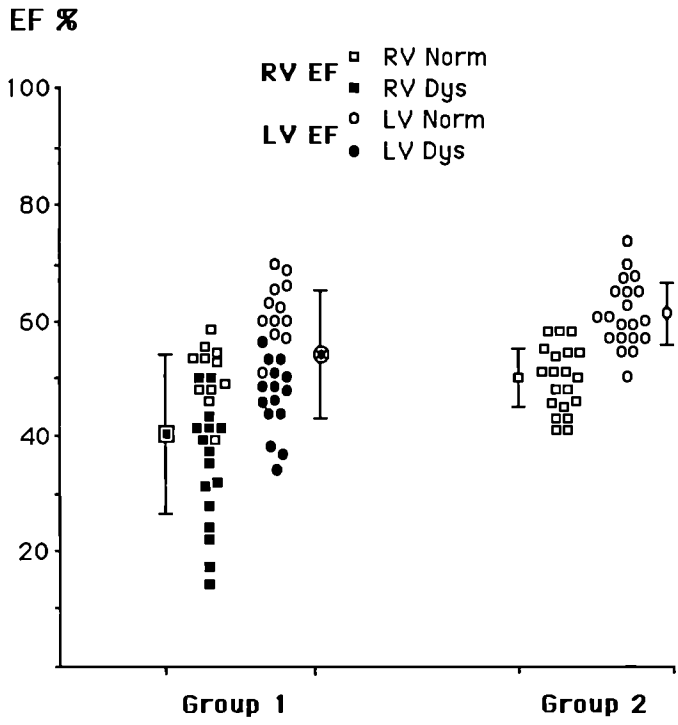


Figure 2: Right and left ventricular ejection fraction (EF) values (individual and mean ± sd values) are represented patients with ventricular arrhythmias (group 1) and in normal controls (group 2). RV, LV Norm=normal right, left ventricular wall motion; RV, LV Dys = right, left ventricular dyssynergies (WMI ≥ 2).
Both right ventricular (RV) and left ventricular (LV) ejection fraction values in group 1 were significantly lower than in group 2 ($p<0.01$).

Correlation between non-invasive and invasive data

Regional ventricular dysfunction was confirmed by contrast ventriculography in 13/24 cases (54%). However the agreement between the non-invasive and angiographic evaluation was significant for the left ventricle ($p<0.05$) but not for the right ventricle. Slightly abnormal hemodynamic measurements were found only in 29% of cases.

The analysis of right ventricular endomyocardial biopsies, performed according to very restrictive criteria, showed abnormal histologic findings in 62% of patients. Both the prevalence and the type of histologic abnormalities of the myocardium were similar to those documented in patients with claimed dilated cardiomiopathy.

Fellow-up results

The follow-up data of 18 patients in the study group were analysed for a mean period of 2.3 years (range 1-5 years). In 8/18 cases (44%) no change of echocardiographic and clinical patterns was documented in a mean follow-up time of 1.7 years (range 1-3 years). By contrast 10/18 patients (56%) showed functional and/or clinical progression of the myocardial disease. In particular 3 patients developed dyspnea (class III NYHA) and 1 patients died suddenly.

Conclusions

Non-invasive techniques are valuable clinical tools to document subclinical myocardial disease in patients suspected of early cardiomyopathy. 2D-Echocardiography and Radionuclide Angiography appear complementary in the evaluation of ventricular function. While echocardiography is particularly usefull in a preliminary screening of patients, the scintigraphic approach allows a better evaluation of the right ventricle and an accurate extimation of possible global systolic dysfunction. Both techniques can also show progression of early myocardial damage. The invasive study can confirm non-invasive findings but seem to add nothing in patients with early cardiomiopathy. It could be recommended when a clinical and/or functional progression of the disease is evident.

If the progression of early myocardial dysfunction in patients with arrhythmias could finally lead to congestive heart failure cannot be stated at present. However it is now clear that complex ventricular arrhythmias are infrequently an isolated manifestation of heart disease; in these subjects an early non-invasive diagnosis of myocardial impairment and a prolonged follow-up is recommended.

IV. Objectives for the next reporting period:

In the next reporting period, a non-invasive evaluation of myocardial functional reserve will be made in patients with early cardiomyopathy. Moreover some possible pathophysiologic mechanisms which could explain both arrhythmias and early myocardial dysfunction will be explored by non-invasive approaches. In particular the coronary reserve will be studied by Positron Emission Tomography and the Autonomic Nervous Control evaluated.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

C.N.R. - Institute of Clinical Physiology of Pisa, Pisa (Italy).

VI. Publications:

Diagnosi e caratterizzazione funzionale di iniziale danno miocardico in pazienti con aritmie cardiache.
D. Neglia, D. Levorato, S. Berti, M. Marzilli, G. Pelosi, C. Marcassa, M.G. Bongiorni, A. L'Abbate, C. Contini.
Cardiologia, 32: 713, 1987.

Holter monitoring in patients with right cardiomyopathy.
C. Arlotta, A.M. Piacenti, D. Levorato, S. Berti, C. Contini.
III Int. Symp. Holter Monitoring, Vienna 1988, pag. 251 (Abstract n. 198).

Myocardial damage and ventricular arrhythmias: an early step toward dilated cardiomyopathy.
C. Contini, S. Berti, D. Levorato, M.G. Bongiorni, M.T. Baratto, C. Arlotta, A.M. Piacenti, A. Pozzolini, L. Paperini, G. Kraft.
Eur. Heart J. 9: 106, Suppl. 1, 1988 (Abstract n. 558).

Cardiac arrhythmias in early and advanced myocardial damage.
D. Levorato, C. Arlotta, S. Berti, A.M. Piacenti, L. Paperini, A. Pozzolini, M.T. Baratto, M.G. Bongiorni, C. Contini.
III Int. Symp. Holter Monitoring, Vienna 1988, p. 135 (Abstract n. 82).

Arrhythmias and arrhytmologenic right ventricular dysplasia: characterization and long-term antiarrhythmic therapy.
D. Levorato, A. Azzarelli, G. Pistelli, C. Contini.
Cardiostimolazione 6: 4, 260, 1988.

Title of the project no.: 2

Diagnostic efficacy of immunoscintigraphy with radioactive monoclonal antibodies.

Head(s) of project:

G. Mariani, Associate Professor of Medical Pathophysiology, University of Pisa.

Scientific staff:

R. Bianchi, M.D., C.R. Bellina, M.D., R. Guzzardi, D. Phys., C. Rosa, M.D., N. Molea, M.D.

I. Objectives of the project:

1) To evaluate the patients exposure to ionizing radiation involved by by the use of a newly developed diagnostic procedure, that is, radioimmuno-scintigraphy of tumor lesions by means of radiolabeled anti-tumor monoclonal preparations.

2) To assess the diagnostic efficacy of this novel medical procedure as compared with a conventional non-ionizing technique (bidimensional ecography).

II. Objectives for the reporting period:

To compare the diagnostic sensitivity of radioimmunoscintigraphy (RISG) with other conventional noninvasive techniques (x-ray) in patients with malignant melanoma.

III. Progress achieved:

The data-base for over 300 melanoma patients included in a multi-center clinical trial on the diagnostic efficacy of RISG was reviewed, and a group of 46 patients was selected for the present evaluation. F(ab')2 fragments from the monoclonal antibody 225.28S has been used for the clinical trial, upon radiolabelling with either Indium-111 or Technetium-99m. The results of RISG were compared with those of conventional x-ray examinations, in terms of patients' classification as to the presence of disease.

Selection of patients for this study was based on the ascertained presence of noncutaneous melanoma lesions all either smaller/equal or larger than a given pre-set size limit, and all proven independently from RISG and standard x-ray examinations. Thus, diagnosis and sizing of the tumor lesions has been based on a combination of physical findings, surgical exploration, transmission CT and ultrasound examinations.

Out of the 46 patients selected for this analysis, 26 were affected by choroidal melanoma, 14 by lung and 6 by axillary lymph node metastases. Given the different clinical meaning at different tumor sites as concerns the possibility of achieving an early diagnosis, the threshold size limit was set at 1 cm for the patients with choroidal melanoma and at 2 cm for all other patients.

Due to the selection criteria given above, the results of each test (RISG or x-ray) could only be either true positive or false negative (in terms of patients' classification). The statistical evaluation of each test was therefore performed solely in terms of sensitivity with respect to the presence of tumor; this was followed by chi-square analysis, to assess the diagnostic sensitivity of RISG as compared to that of x-ray examination.

In the overall population that forms the selected data-base for the present study, the diagnostic sensitivity of RISG resulted to be

82.6% (or 38/46), significantly higher than that observed for x-rays (58.7%, $p < 0.025$). In the choroidal melanoma group, RISG performed significantly better than x-ray both for all patients considered altogether (80.8% versus 50%, $p < 0.05$) and, particularly so, for the patients with melanoma lesions 1 cm (63.6% versus 9.1%, n=11, $p < 0.025$). Whereas, the difference was not statistically significant for patients with lesions $>$ 1 cm (93.3% for RISG versus 80% for x-ray).

Due to the small number of patients in each group, patients with lung metastases and patients with axillary lymph node metastases were analyzed as a single group. Diagnostic sensitivity of RISG resulted to be higher (though not reaching statistical significance) than that of x-ray in this series of patients when considering either the entire group regardless of tumor lesion size (85% versus 70%) or the patients with lesions $>$ 2 cm (100% versus 57.1%, n=7). Whereas, in the patients with tumor lesions $\leq$ 2 cm RISG sensitivity was identical to that observed with x-ray (76.9%).

The results of this analysis confirm the remarkably good diagnostic performances of tumor RISG as compared to conventional x-ray, particularly in the choroidal melanoma group and in the patients of this group with the smallest tumor lesions. This finding is of crucial importance as to the possibility of achieving an early diagnosis of primary and/or recurrent cancer.

IV. Objectives for the next reporting period:

1) To perform tissue distribution kinetic studies in tumor patients following the administration of various RISG agents, such as anti-adenocarcinoma B72.3-IgG1, B72.3-F(ab')2, etc.

2) To calculate the internal radiation dose burden to patients following the administration of various RISG agents, by feeding the kinetic tissue distribution data in the CAMIRD III software package.

3) To extend the analysis of RISG sensitivity in terms of both actual number of patients studied and comparison of RISG with other noninvasive diagnostic techniques.

4) To perform a comparative cost/benefit analysis of RISG and other noninvasive diagnostic technique.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

1) SORIN BIOMEDICA; Saluggia, Vercelli (Italy).

2) Institute of Radiology of the University of Pisa, Pisa (Italy).

3) Center of Nuclear Medicine of the University of Pisa, Pisa (Italy).

4) C.N.R. - Institute of Clinical Physiology of Pisa, Pisa (Italy).

VI. Publications:

Diagnostic efficacy of tumor radioimmunoscintigraphy with monoclonal anti-CEA F02305-F(ab')2 versus standard x-ray and ultrasound.
G. Mariani, C. Rosa, L. Donato.
Eur. J. Nucl. Med., 14:5/6,223, 1988 (Abstract n. 4).

Internalization of monoclonal 3G5-IgM by rat insulinoma cells.
G. Mariani, A.D. Van den Abbeele, C.N. Venkteschan, A. Kaldany, S. Ito, S.J. Adelstein, A.I. Kassis.
Eur. J. Nucl. Med., 14: 5/6, 252, 1988 (Abstract n. 177).

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-132-

CEPN-INSERM Unité 240
Centre d'Etude sur l'Eval.de la
Prot.dans le domaine Nucléaire
B.P. 48
F-92263 - Fontenay-aux-Roses Cédex

Head(s) of research team(s) [name(s) and address(es)]:

Dr. F. Fagnani
CEPN-INSERM Unité 240
B.P. 48
F-92263 - Fontenay-aux-Roses Cédex

Telephone number: 01/46.54.74.67

Title of the research contract:

Analysis of the patient exposure to radiation from medical diagnosis: exposure data and quality assurance.

List of projects:

1. Assessment of the somatic dose related to medical radiodiagnosis.
2. Quality assurance in medical diagnostic radiology.

Title of the project no.:

ASSESSMENT OF THE SOMATIC DOSE RELATED TO MEDICAL RADIODIAGNOSIS AND QUALITY ASSURANCE.

Head(s) of project:

MACCIA C.

Scientific staff:

BENEDITTINI M., FAGNANI F., LEFAURE C., MACCIA C.

I. Objectives of the project:

The final objectives of the project are :

- to complete previous evaluations of the absorbed dose due to diagnostic radiology procedures in France in order to estimate the collective risk associated with the use of ionizing radiation in medicine ;

- to study the total population exposure due to the CT examinations by using a common dosimetric methodology elaborated together with the GSF (Germany), the NRPB (UK), the USL n°7 (I) and the CEPN (F) ;

- to contribute to the establishment of quality criteria for diagnostic radiographic images.

II. Objectives for the reporting period:

- To analyze statistical data gathered in the multinational CEC Trial conducted in 1988 (project 1).

- To handle CT dose measurements carried out on 15 machines operating in the PACA (Provence-Côte d'Azur) region (project 2).

III. Progress achieved:

PROJECT N° 1

In 1987 a Study Group of the Radiation Protection Programme of the Commission of the European Communities (CEC), initiated a project on the establishment of quality criteria for diagnostic radiographic images.

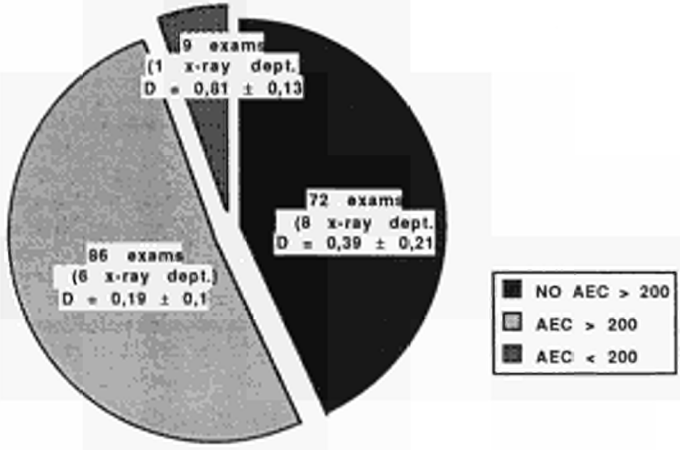
The main goal of this project was to provide practitioners with a provisional acceptable list of both radiological and technical requirements (including dose values) which could be useful to judge the quality of the radiographs routinely undertaken in diagnostic radiology while keeping the patient received dose as low as reasonably achievable.

A questionnaire was therefore circulated to 24 different European x-ray departments which have activily participated in the project by checking, for each examination type (chest, skull, lumbar spine, pelvis and sacrum, urinary tract, breast), the suggested image quality and commenting on their relevance.
More than 900 x-ray examinations performed in 10 European countries were studied.
As far as the dosimetry is concerned, Entrance Surface Dose (ESD) was measured for each examination type and for each projection by sticking a thermoluminescent dosimeter chip on the x-rayed patient's skin.
On the basis of a "medical scoring system" defined by a group of radiologists, each radiographic projection was assessed in order to select the "most efficient" way of performing the examination.

Considering the radiological equipment and the film/screen sensitivity classes, four categories were defined in order to compare results obtained with so many different radiological units.
Basically, discrimination was made between the x-ray tables equipped with an Automatic Exposure Control system (AEC) and those manually operated. Concerning the film/screen sensitivity classes, attention was paid to the CEC document requirements, namely : minimum sensitivity class of 200 for the chest.

An example of the results obtained is given in Figure 1 for the chest examination.

SELECTING THE MOST EFFICIENT TECHNIQUE (CHEST P/A)



Implementing the selecting procedure led to keep 167 high image quality score chest x-rays out of 208 acceptable films corresponding to three groups of technique shown in the "chest pie" chart.

First of all, no people were found to work exclusively manually and using a low sensitivity film/screen class, and very few examinations were carried out using low sensitivity films with the AEC system actually installed (1 x-ray department).

The great majority of the selected high image quality score films were taken either with an operating AEC system or with a manual operating equipment but always with sensitivity film class above 200. For these two categories of technique dose hierarchy was respected with an average figure of 0.19 mGy (AEC system) and 0.39 mGy (without AEC system) respectively. This clearly shows the strong impact of the AEC techniques in improving dose reductions when the appropriate film/screen combinations are used and, at the same time, the relevance of the image quality criteria defined in the CEC trial.

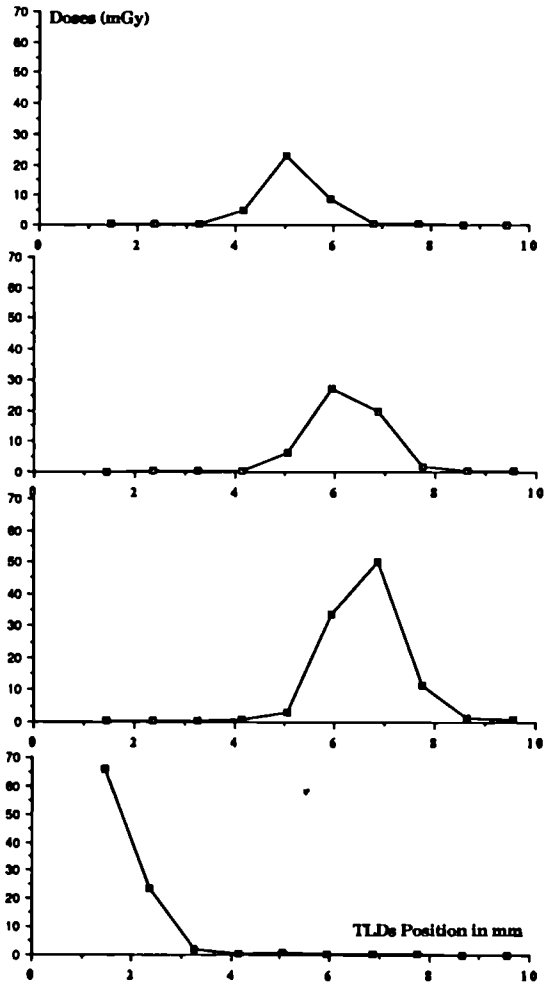
PROJECT N°2

To allow for variations in x-ray tube output, x-ray spectrum, beam collimation and scanning geometry between different CT scanners and between different selections of the scanning parameters on a particular machine, the free-in-air axial dose profile for a single slice at the centre of rotation was measured for each commonly used set of scanning paramters (kVp, mAs, filter, slice thickness, etc) on 15 scanners installed in the PACA region. A row of TLDs spaced at approximately 1mm intervals were aligned along the axis of rotation with the central TLD as close as possible to the centre of the scanned slice.

This provided relevant informations on the adequacy of the beam collimation on CT scanners which is quite variable from one design to another and can seriously influence patient dose.

Figure 2 compares the dose profiles measured for 4 different machines of the same model (CE 10 000) operating under routine conditions (130 Kvp ; 6,8 (s) scan time ; 2 mm slice thickness).

COMPARISON OF DOSE PROFILES FOR 4 DIFFERENT CT SCANNERS.



As far as the free-in-air dose integrated over the slice thickness is concerned, figures obtained have shown large discrepancies between CT machines considered. For instance, for a 1 mm slice thickness, 130 kVp and 6,8 (s) scan time, dose were found to vary from 108 mGy to 340 mGy with an average value of 130 mGy.

All these data are being stored in a computer file in order to evaluate, through the Monte-carlo dose coefficient factors provided by the NRPB, the effective dose equivalent associated to the CT examination.

Experimental dose measurements will be combined with the frequency of CT examinations yearly performed in France. To get a national collective dose figure attributable to this kind of radiological practice.

IV. Objectives for the next reporting period:

To extend the experience gathered in this trial to the pediatric radiology. To initiate the process of establishing both image quality criteria and technical requirements for fluoroscopy and digital radiology.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

NRPB
National Radiological Protection Board - Chilton Didcot - Oxfordshire OX11 ORQ

GSF
Institut für Strahlenschutz - Ingolstädter Landstr. 1 - München - NEUHERBERG

USL n°7
Servizio di Fisica Sanitaria - Ospedale "Maria della Misericordia" Via Pieri - UDINE

VI. Publications:

C. MACCIA, BF. WALL, R. PADOVANI, P. SHRIMPTON, B. HUSSON, "Results of a trial set up by a Study Group of the radiation protection Programme of the CEC", to be published Brit. Inst. Jl

B. HUSSON, "Contribution à la recherche des critères de qualité d'images d'un cliché radiographique", DEA d'Imagerie Médicale, Université de Paris XI, Décembre 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-315-UK

Northern Regional Health Authority
Northern R.H.A. Headquarters
Benfield Road, Walkergate
GB - Newcastle upon Tyne NE6 4PY

Head(s) of research team(s) [name(s) and address(es)]:

Dr. K. Faulkner
Regional Medical Physics
Newcastle General Hospital
Westgate Road
GB - Newcastle on Tyne NE4 6BE

Telephone number: 091/2738811

Title of the research contract:

Automated quality assurance and patient dosimetry in diagnostic radiology.

List of projects:

Automated quality assurance and patient dosimetry in diagnostic radiology.

Title of the project no.:

Automated Quality Assurance And Patient Dosimetry In Diagnostic Radiology.

Head(s) of project: Dr K Faulkner

Scientific staff: Miss C L Chapple
Dr R M Harrison
Mr P Hedley
Mr C J Kotre

I. Objectives of the project:

a) To design and develop a computerised method of automatically monitoring tube and generator parameters to perform online quality assurance and radiation dosimetry.

b) To determine the benefits (quality of diagnostic information and exposure reduction) for the patient due to the introduction of automated quality control.

II. Objectives for the reporting period:

a) To identify the relevant tube and generator parameters to be monitored.

b) To commence development of appropriate measurement techniques.

c) To review published normalised organ dose data.

d) To assess the most appropriate interfacing and data processing methods for the instrument and X-ray generator control console.

III. Progress achieved:

An X ray unit has been installed in the Regional Medical Physics Department. Modifications have been made to the three phase power supply to the X ray room to accommodate the power requirements of the X ray generator.

Electrical-mechanical and radiation protection commissioning tests have been undertaken on this unit.

A preliminary study into the data handling and processing requirement of the microcomputer to be used to monitor various tube and generator parameters has been completed. Figure 1 is a schematic diagram of the X ray unit, the microcomputer and the data input into the microcomputer. Data will be input by four basic methods:-

i) directly by the operator via the keyboard.

ii) by decoding of signals from within the microprocessor controlled X-ray generator console.

iii) by decoding of signals carried along an RS232 interface.

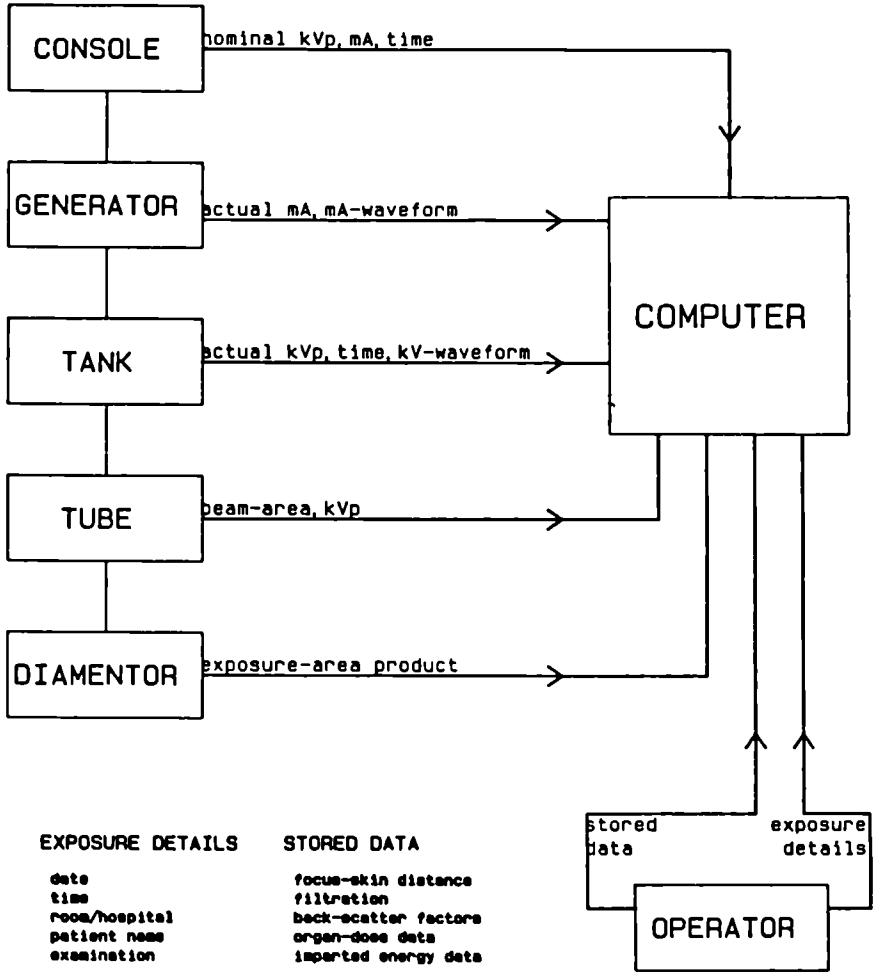
iv) by analysis of signals produced, when voltages are fed into an analogue to digital convertor.

Special consideration has been given to the number of digitization channels, digitization speed required and computer software needed to control the analogue to digital convertor. A sixteen channel, twelve bit resolution, with up to 6kHz sampling rate, analogue to digital convertor has been identified and purchased.

A light beam diaphragm assembly attached to the X-ray tube is being modified to enable the direct measurement of field size of the X-ray collimators. These modifications are currently being constructed in the Department's Mechanical Workshop. A Diamentor exposure area product ionisation chamber will be attached to the light beam assembly. The possibility of controlling the Diamentor via an RS232 link to the computer is being investigated. The position of the precision potentiometer on the X-ray transformer and mA link on the X-ray generator have been identified. Appropriate wiring and cable trunking have been installed. Preliminary work has been performed on decoding of information within the microprocessor controlled X-ray generator console. A method of automatically monitoring the focus skin distance using an ultrasonic distance rule is being investigated.

Consideration has been given to the size, scope and organisation of the database to contain the quality assurance and patient dosimetry information. A commercially available, integrated software package capable of modification to meet these needs has been identified and purchased. Special emphasis has been placed on the man machine interface with the intention of making this aspect of the instrument as user friendly as possible.

BLOCK DIAGRAM FOR SYSTEM



IV Objectives for the next reporting period

a) To further develop and implement a prototype instrument.

b) To evaluate the potential of the instrument for performing on-line quality assurance using phantom studies.

c) To perform a preliminary survey using patient equivalent phantoms to assess the potential of the instrument on the reduction of patient doses.

d) To establish links with other research groups, particularly in the fields of paediatric radiology, mammography and digital fluorography.

V Other research groups collaborating actively on this project:

None.

VI Publications:

None

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-211-D

Ludwig-Maximilian-Universität
Kinderklinik
Röntgenabteilung
Geschwister-Scholl-Platz 1
D-8000 - München 22

Head(s) of research team(s) [name(s) and address(es)]:

Dr. H. Fendel
Röntgenabteilung
Dr. von Haunersches Kinderspital
Lindwurmstrasse 4
D-8000 - München 2

Telephone number: 089/5160.3102

Title of the research contract:

The Principles and the Practicability of Quality Control and Quality Assurance in Paediatric Radiology.

List of projects:

1. The Principles and the Practicability of Quality Control and Quality Assurance in Paediatric Radiology.

Title of the project no.:

The Principles and the Practicability of Quality Control and Quality Assurance in Paediatric Radiology.

Head(s) of project:

Dr.med. Helmut Fendel, Leitender Akademischer Direktor

Scientific staff:

Dr.med. K.Schneider Dr.med. C.Bakowski, Dr.med. M.Kellner, Dr.med. S.Burtscher, Dipl.Psych. M.M.Kohn, MTRA K.Schweighofer, cand.med. J.Pehe, cand.med. M.Weisbach, cand.med. M.Zeiler, cand.med. M.Hösle, cand.med. R.Pichlmaier, cand.med. H.Maul

I. Objectives of the project:

The project has the objective to screen and assess problems related to radiation protection in paediatric radiology. Optimization, quality control, and quality assurance of radiological imaging studies of newborns, infants, and children are different from those in adults. They are, however, mandatory in terms of radiation protection of the public because they concern the most sensitive part of the general population. The objective of the project is to survey how individual optimization measures can be effective in daily routine and to what extent they are practicable with the final goal of establishing standards for quality control and quality assurance in paediatric radiology.

II. Objectives for the reporting period:

As was planned, the first survey was finished during the reporting period. A detailed questionnaire was used to survey the personnel, structural and technical resources of all paediatric radiological diagnostic centers of the Federal Republic of Germany including West Berlin which are headed by a qualified paediatric radiologist. Phantom measurements simulating some typical paediatric radiologic examinations in these centers followed. Meanwhile, the second part of the survey was started, which includes the collection of similar data at other roentgen diagnostic centers, which are not headed by an qualified paediatric radiologist, but occaissionally examine children. The third stage of this study, i.e. the survey and measurements in paediatric private praxis has also been started. The acquired knowledge of the actual conditions under which roentgen examinations in children are performed will serve as a basis for the proposed recommendations and guidelines.

III. Progress achieved:

The questionnaire surveys 1) the type and size of the clinic or department for which this diagnostic center works for and the yearly number of examinations, 2) the number and professional training of the personnel providing these services, 3) the number and size of the rooms available for this purpose, 4) the number and type of technical equipment, 5) the number and type of imaging receiving systems, 6) the number and type of special equipment available for the examination of non-cooperative children and the radiation protection of the patient, 7) specifications of the dark room and the equipment for film development and 8) details on how some typical roentgen examinations of children (skull ap/pa, lateral spine, abdomen ap supine, thorax ap/pa, infant hip for CDH) are performed. The measurements which follow this survey simulate the exposures of the skull, spine, abdomen and thorax of a 10 month old infant, of the infant hip for CDH of an 4 month old infant, and of a thorax exposure ap with a mobile unit of a newborn weighing 1,000 g in intensive care. The usual settings of the study center are simulated and an adapted phantom is used. The radiation beam is measured for 1) actual exposure time, 2) dose rate during the exposition, 3) course of wave length of the radiation, and 4) the total effective radiation filtration. This data are used to calculate the 1) patient skin dose, 2) dose at the image receptor, and 3) dose yield per mAs. X-rays of a test plate allow for a check of cassette fitting, optical density, image contrast, resolution, beam collimation, centering etc., as well as utilization of film sensitivity by the development.

By the end of 1988, these measurements have been made at 35 different study centers. Although all of these centers are under the supervision of a qualified and experienced paediatric radiologist, the analysis of the data shows that there are major differences between and within the individual centers. Further analysis will allow for conclusions as to the cause of these differences. In addition, the special difficulty of maintaining constancy in the exposure conditions for the short exposure times and the respective low dose ranges typical for paediatric radiology has been clearly shown. These first results of the study have been presented at the CEC Workshop "Optimazation of Image Quality and Patient Exposure in Diagnostic Radiology", Oxford 27. - 29.9.1988.

IV. Objectives for the next reporting period:

The results of this first series of surveys and measurements has to be carefully analyzed. The inclusion in this study of those roentgen diagnostic centers which are not headed by a paediatric radiologist and which are not adapted to the special requirements of paediatric radiology as well as data from private praxes of paediatricians, radiologists, orthopedists and urologists should continue. In addition, a work group consisting of European paediatricians and medical physicists will develop quality criteria for paediatry and start dose measurements for some typical paediatric X-Ray examinations throughout Europe by the end of 1989.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr.Drexler, Gesellschaft für Strahlen- und Umweltforschung, Neuherberg/München

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-299-P

Laboratorio Nacional de Engenharia
e Tecnologia Industrial (LNETI)
DPSR - Azinhaga dos Lameiros
Estrada do Paço de Lumiar
P-1699 - Lisboa

Head(s) of research team(s) [name(s) and address(es)]:

Dr. J.P. Galvão
DPSR
LNETI
Estrada Nacional 10
P-2685 - Sacavem

Telephone number: (1)255.00.21

Title of the research contract:

1. Dose assessment and quality assurance in diagnostic radiology.

List of projects:

1. Dose assessment and quality assurance in diagnostic radiology.

Title of the project no.:

Dose assessment and quality assurance in diagnostic radiology

Head(s) of project:

J.P. Galvão

Scientific staff:
J. Vaz Carreiro, A. Ferro de Carvalho, Rui Serro, Estela Amaral, Paula Rocha, João Alves, Augusto Oliveira.

I. Objectives of the project:

Assessment of patient doses to the portuguese population in order to have an overview of the radiological practices and the study of its improvement by application of quality assurance measures to reduce unnecessary doses to patients.

II. Objectives for the reporting period:

- selection of the most significant regions taking into account the frequencies of radiographic examinations.
- exposure measurements in selected public hospitals considering their ranking in the National Health Service.
- acquisition and understanding of the operation of the NEXT computer program.
- inventory of dental, mammographic and CT equipments.
- survey of mammographic and dental installations.
- development and test of methods and devices to be used in the survey of mammographic and dental installations.

III. Progress achieved:

Population doses assessment

1 - Methodology

The methodology applied is based on the NEXT programme. The exposure measurements are performed with ionization chambers Victoreen 660 model on phantoms in the premises. Public hospitals are surveyed considering the frequency of radiological examinations.

2 - Results

First tests for exposure measurements were performed at one of the main Lisbon's public hospitals in order to establish the adequate techniques and to provide training in the field to the project staff.

Studies were performed with the portuguese Statistics Institute for the evaluation of the national frequencies of all types of radiological examinations within each region and a methodology was set up for evaluating their statistical representativeness.

A software consultant was commited for the conversion of the NEXT computer program to the Department's personal computers.

A data collection programme was carried out in order to obtain the physical parameters and technical specifications of X-ray tubes and exposure measurements in phantoms. Thirty one public hospital premises have been visited and one hundred X-ray tubes were surveyed which represent two thirds of the total field work.

3 - Discussion

Field work is fairly advanced considering the data collection scheduled.

Due to technical difficulties found by the consultant to complete the conversion of the NEXT programme to PC it was not possible until now to proceed the necessary tests.

Quality assurance

1 - Methodology

National inventory of dental, mammographic and CT

equipments is carried out using several sources of information (licensing board, dealers of films and X ray equipments, radiologists and practitioners associations).

To carry out the mammography survey a DPSR staff team visits each installation to collect technical information concerning the X-ray equipment, film screens, cassettes, AFP, etc.

Test and measurements are carried out for the radiation output, AEC performance, beam alignment, image quality, film processing quality, and some viewing conditions (illumination).

In what concerns the dental survey, two kits and a questionary are sent by post to each installation to collect data about radiographic techniques, entrance exposure, X-ray equipment and image quality.

Dose measurements are performed with lithium fluoride dosemeters.

2 - Results

Inventory of mammographic and CT equipment was finished and a mailing list of dental installations was prepared.

Development and test of methods and devices were carried out to be applied in mammography, dental surveys and in the quality control programme.

Survey of mammographic equipment was done in 50% of all installations in the country.

3 - Discussion

Some difficulties arose in the performance tests of dental kits determining changes in the activities scheduled for the last three months. Thus the start of the dental survey was postponed to 1989.

The mammographic survey was delayed in order to permit the implementation of the quality control programme in some selected installations. The survey is foreseen to be finished in the first quarter of 1989.

IV. Objectives for the next reporting period:

- Completion of field work for exposure measurements.
- Data processing with the NEXT programme.
- Dose assessment.
- Conclusion of dental, mammographic and CT installations surveys.
- Conclusion of the quality control programme for mammography installations.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- Direzione Sicurezza Nucleare e Protezione Sanitaria - ENEA
  Via Vitaliano Brancati 48
  ØØ144 Roma

- Instituto Superiore di Sanitá - Physics Laboratory
  Viale Regina Elena 299
  ØØ161 Roma

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BI6-F-133-D

Head(s) of research team(s) [name(s) and address(es)]:

Prof. Dr. W. Jacobi
Institut für Strahlenschutz
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Dr. G. Drexler
Institut für Strahlenschutz
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Telephone number: 089/31.87-2241

Title of the research contract:

Analysis of exposure in radiology

List of projects:

1. Analysis of exposure in radiology

Title of the project no.:

BI6-F-133-D

ANALYSIS OF EXPOSURE IN RADIOLOGY

Head(s) of project:

Dr. G. Drexler

Scientific staff:

Dr. D.F. Regulla
Dipl. Math. M. Zankl
Dr. G. Drexler
Dipl.-Phys. W. Panzer
Dr. N. Petoussi
Dipl.-Phys. R. Veit

I. Objectives of the project:

- Collection of dose values under routine conditions; performance of field studies in radiodiagnosis.
- Development of realistic mathematical phantoms from computer tomograms for the Alderson Rando phantom for babies, children and adults; production of CT data files.
- In therapy, calculation of organ and tissue doses outside the target region from standardised and realistic mathematical phantoms and patients.
- Tentative quantification of radiological risk.

II. Objectives for the reporting period:

- Completion of the construction of a mathematical phantom from the CT data of the physical Alderson phantom; performance of dose calculations for selected volumes within the Alderson phantom and comparison with corresponding measured doses.
- Continuation of construction of mathematical phantoms from human CT data with priority to adults; comparison of doses as achieved from the CT and MIRD-5 type mathematical phantoms.
- Continuation of the analysis of organ doses in radiation diagnostics and therapy.
- Field quality checks of the speed of dental X-ray films, and of film processing procedures in dental practices.

III. Progress achieved:

Comparison of calculated and measured doses: An Alderson-Rando phantom containing a series of TL dosemeters was scanned in a CT- machine from head to bottom. From these data a three-dimensional mathematical Alderson voxel model was reconstructed. In this model dosemeter volumes were defined at the same positions at which TL dosemeters were positioned in the real phantom. The objective was to evaluate on the degree of agreement between measured and computed doses.

The dose measurements resp. calculations were performed in 28 target volumes of the Alderson phantom, resp. its mathematical reconstruction. For the TL measurements, a dosimetric set of 2 or 6 TL detectors in PVC cylinders were prepared fitting the holes in the Alderson slices. The TL detectors were individually calibrated, by means of ionisation chambers, under exposure conditions in the phantom.

For the mathematical reconstruction as well as for the conversion of doses measured by TLD into average absorbed dose in the target volumes, the chemical composition and density of the various Alderson tissues had to be defined.

Irradiation conditions were chosen as follows: (a) Whole body CT from which examination the mathematical model has previously been constructed, (b) Co60 whole body irradiations a.p. and p.a. and (c) some common X-ray examinations (skull, thorax, abdomen p.a. and a.p.). Results are given in Table 1.

Table 1: Results on differences between measured and calculated absorbed dose values in the target volumes per unit dose free in air in the focus-to-skin distance or free in air on the axis of rotation (CT) for whole body irradiations

	CT	Co60 p.a.	Co60 a.p.
Range of differences	-10 %/+15 %	-10 %/+15 %	-15 %/+13 %
Differences within ± 10 %	93 %	85 %	80 %
Average difference	0.2 %	1.7 %	- 5.0 %
Stand. dev. of differences	5.9 %	7.1 %	6.3 %

Mathematical phantom files: The construction of a mathematical phantom from the whole body CT data of a twelve years old boy was started. This boy is a leukemia patient who was scanned before he was to undergo a total body irradiation and a bone marrow transplant.

It was not yet possible to obtain a whole body CT scan of an adult with contiguous slices, as necessary for the construction of a mathematical voxel phantom of acceptable resolution.

Analysis of organ doses: As part of a common study of several CEC contractors on the determination of organ doses caused by CT examinations, Monte Carlo calculations have been performed with the mathematical phantoms "Adam", "Eva", "Child" and "Baby". In these calculations the exposure conditions most common in the FRG were simulated, namely a 360° rotation of the fan beam, a distance of 76 cm from the focus to the axis of rotation, a tube voltage of 125 kVp and filtrations of 2.2 mm Al + 0.2 mm Cu and 2.2 mm Al + 0.4 mm Cu, respectively. The calculations were performed as contiguous single slices of 10 mm thickness from the bottom of the trunk up to the top of the head for the adult phantoms and as contiguous single slices of thickness 8 mm and 4 mm from the toes up to the top of the head for the seven year old child and for the eight week old baby, respectively. The results were sent to NRPB where similar calculations for differing irradiation conditions have been performed. The different data sets will be combined to provide a common data basis for all contractors.

Using the CT phantom of a seven year old child, organ doses resulting from various examinations in pediatric radiology were assessed. The irradiation parameters for the organ dose calculations were selected from examinations of the age group 5 to 7 years.

Results of these calculations were compared to organ doses compiled by Rosenstein et al. in 1979 for pediatric radiology. These organ doses were calculated using MIRD-type children phantoms. The exposure conditions considered by Rosenstein et al. are common in the USA and are somewhat different from those simulated in the present calculations with regard to field size, focus-to-film distance and photon spectra.

Doses for organs well inside the beam did not differ greatly in this comparison. As expected, the higher discrepancies occurred for doses to organs near the edges of the fields which were in the beam in one of the calculations and outside in the other. Differences in the red bone marrow doses were expected due to the entirely different modelling of this tissue in the phantoms considered. Results of this study were presented at the CEC workshop on "Optimisation of Image Quality and Patient Exposure in Diagnostic Radiology", Oxford, September 1988.

Using the adult mathematical phantoms Adam and Eva, the effect on organ doses due to the change of tube voltage and focus-to-film distance was studied. For these parameters the minimum and maximum values as recommended in the CEC draft document "Quality Criteria for Diagnostic Radiographic Images" were considered. It was found from these calculations that for single organs dose differences up to 40-50 % can be observed resulting from the same type of examination, even when the technical parameters are within the recommended range. Results of this study were also presented at the CEC workshop in Oxford.

In many fields of radiology, and in particular in radiotherapy, dose measurements at specified depths in water phantoms or in other tissues have to be performed, mainly for calibration purposes. In order to properly interpret the dosemeter readings, the energy spectrum at the place of the dosemeter has to be known. A study has been started in order to determine the photon spectra at different depths inside water phantoms. For that purpose, Monte Carlo techniques are used and emphasis is given on the effect of the beam and phantom size.

Quality checks in dental radiology: Film developing was checked by personal visits of around 100 dental practices in Munich using pre-exposed films and a questionnaire on the technical details of applied procedures. For completion, notes were taken from the visual inspection. Further, patient dosimetry was performed using TL detectors positioned, in the beam, on the skin (entrance dose) and on the film. The beam diameter was controlled by a Röpak set. As a preliminary result film developing procedures and operational behaviour of the radiographer still seem to differ remarkably from practice to practice; details are going to be evaluated.

IV. Objectives for the next reporting period:

- Completion of the comparison between dose values in a physical Alderson Phantom and values calculated in its mathematical reconstruction.

- Continuation of construction of mathematical phantoms from human CT data with priority to adults; comparison of doses as estimated from the CT and MIRD-5 mathematical phantoms.

- Continuation of the analysis of organ doses in radiation diagnostics and therapy.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. Fendel and his Staff, University Children's Clinic, Munich, W-Germany

VI. Publications:

Panzer, W., Scheurer, C., Drexler, G.: Feldstudie zur Ermittlung von Dosiswerten bei der Computertomographie. Fortschr. Röntgenstr. 5, 534-538 (1988)

Petoussi, N., Zankl, M., Williams, G.*, Veit, R., Drexler, G.: Organ doses from radiotherapy for cervical cancer using Monte Carlo techniques. Dosimetry in Radiotherapy, IAEA, Wien, Österreich, ISBN 92-0-010088-0, 293-295 (1988)

Zankl, M., Veit, R., Williams, G.*, Schneider, K.**, Fendel, H.**, Petoussi, N., Drexler, G.: The construction of computer tomographic phantoms and their application in radiology and radiation protection. Radiat. Environ. Biophys. 27, 153-164 (1988)

*Medical College of Ohio, Toledo, USA
**Dr. von Haunersches Kinderspital, Univ. München

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-134-IRL

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Voluntary Hospitals
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Head(s) of research team(s) [name(s) and address(es)]:

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Dept of Med. Phys.& Bioeng.
St James's Hospital
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IRL - Dublin 8

Telephone number: 01/532.385

Title of the research contract:

Specification of uniformity and noise limited exposure reduction in radiological image intensifier - TV systems as an adjunct to quality control and optimization of exposure.

List of projects:

1. Specification of uniformity and noise limited exposure raduction in radiological image intensifier - TV systems as an adjunct to quality control and optimization of exposure.

Title of the project no.: Specification of uniformity and noise limited exposure reduction in radiological image intensifier TV systems as an adjunct to quality control and optimization of exposure.

Head(s) of project: J. F. Malone.

Scientific staff: P. Cooney,
W. van der Putten.

I. Objectives of the project:

(a) Development of parameters to be included in methods of specification, acceptance testing and quality control in Radiological Systems based on new technology.

(b) Reduction of the dose required in individual examinations through correct specification of exposure levels required to meet particular imaging needs.

(c) Optimization of the use of medical exposure through a clear understanding and specification of how particular measures of image quality are achieved with digital systems.

(d) Assessment of requirements in practice to allow Image Quality Criteria proposals be implemented in one large department.

II. Objectives for the reporting period:

(a) Both system and component uniformity will be investigated further by extending the survey to cover up to twenty X-Ray/II-TV Systems.

(b) An attempt to model how component uniformities contribute to system uniformity. In addition the model will be tested.

(c) Tolerances will be established for the measurement methods used and associated instrumentation.

(d) Dosimetric studies will be continued.

(e) Influence of Magnification/Matrix Size will be further investigated.

(f) Studies on the noise level/signal to noise ratio in images and subtracted images will be initiated with particular emphasis on the influence of dose/dose rate/automatic controls.

(g) Contact will be continued with other Research Group(s) involved in this area.

III. Progress achieved:

The System and Component Uniformity investigations have been ongoing and progress has been achieved in that there is a grouping of the majority of measurements between the different systems examined. However, with the exclusion of outlying systems there is still a considerable variation between the grouped systems. Nevertheless, it is hoped that limiting values for the measurements will be suggested with the acquisition of more data.

A detailed quantitative and subjective evaluation of the exposure rates and noise levels associated with Automatic Exposure Control (AEC) facilities in use on X-Ray Image Intensifier-TV (XII-TV) Systems has been undertaken. The measurement methods developed have been applied in a pilot study to three different manufacturers' XII-TV Systems. Quantitative studies were carried out by digitising the images using a digital image processor (Quantel, Intellect-100) and measuring the signal to noise ratio (SNR) for the graded levels of x-ray beam attenuation used and the corresponding exposure rates. Exposure rate measurements were made at the entrance plane of the II using a 180cc pancake ion chamber and a dosemeter (MDH 2025). X-Ray beam attenuation was provided by inserting standard size telephone books into the beam, and attenuation thickness is expressed in the results in mm of paper (163mm paper $\approx$ 10cm water). A subjective comparison of image quality between the three systems was also performed using the Leeds Test Objects for noise (TO.N3), contrast detail (TO.10), and resolution (Huttner).

Figure 1 plots the normalised exposure rate, measured with each system, against increasing amounts of beam attenuation. It is clear that in each case the AEC does not hold the exposure rate at the entrance plane to the II at a constant value and that there is considerable difference in the levels of exposure rate between systems.

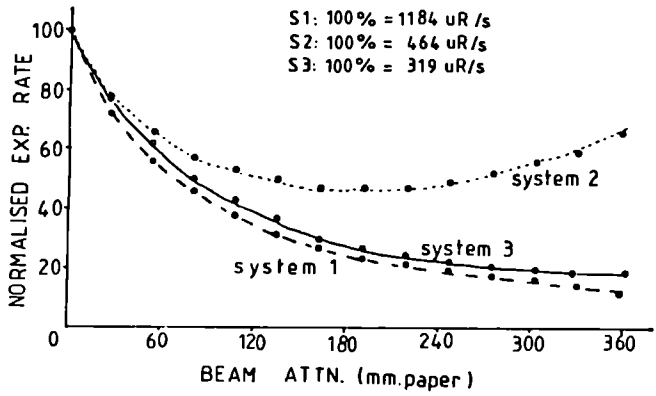

FIG. 1. Relationship between exposure rate and beam attenuation.

With System 1 the measured SNR dropped by a maximum of 4.5 dB for increasing amounts of beam attenuation. This drop was largely dependent on a reduction in the signal level while the measured noise remained relatively constant. With System 2 and 3 the SNR remained relatively constant over the attenuation range examined. This was achieved with each system by different means. System 2 exhibited a drop in signal level for increasing attenuation, with a corresponding drop in the measured noise. System 3 held the signal level constant with only a small increase in the measured noise at the higher attenuation levels.

Despite the variations observed in the SNR measurements very little difference was observed in the noise and contrast detail performance of each system, as assessed using the Leeds Test Objects. For each system the drop in the spatial resolution with increasing attenuation was the same.

In conclusion it has been found that AEC does not hold the input exposure rate at a constant level and that there are wide variations in exposure rates between systems. One system did not maintain a constant SNR while two of the three systems did not maintain a constant image brightness level over the range of exposures examined.

IV. Objectives for the next reporting period:

(a) Uniformity investigations will be continued with a view to suggesting tolerances for the measurement methods used and associated instrumentation.

(b) AEC studies will be further advanced to examine the relationship between the exposure rate and the light output of the II and the influence of automatic gain control in the TV camera.

(c) Studies will be initiated to evaluate a special purpose digital cardiac imaging system with cine (including doseimetric considerations).

(d) The possibility of implementing the EC Image Criteria Document in a working department will be examined.

(e) Contacts with Standards organisations, such as ETCI, (Electro-Technical Council of Ireland) will examine the possibility of implementing proposals like the Image Quality Criteria Document using a Standards approach.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Dr. L. Malone, Principal Physicist, Beaumont Hospital.

Dr. R. Kirkham, Chairman TC-10 (Electromedical Equipment) of Electro-Technical Council of Ireland, and Chairman of TC62 CENELEC Electro-Medical Committee.

Dr. K. Faulkner, Newcastle General Hospital, Newcastle Upon Tyne, U.K.

VI. Publications:

Cooney, P., Maher, K.P., and Malone, J.F., 1988. Measurement of the Uniformity of X-Ray Image Intensifier-TV Systems and of the system components. British Journal of Radiology, Suppl. 23 (in press).

Cooney, P., Malone, L.A., and Malone, J.F., 1988. Exposure Rate and Noise Level Evaluation of Automatic Exposure Control Systems. Suppl. British Journal of Radiology (in press).

Van der Putten, W.J.M., Cooney, P., and Malone, J.F., 1988. Quality Control of Multiformat Cameras: A Practical Approach. Poster Presentation; Book of Abstracts; NRPB/CEC Workshop, Oxford.

Cooney, P., Maher, K.P., and Malone, J.F., 1988. Quantitative Uniformity Analysis in Radiological Image Intensifier-TV Systems using a Digital Image Processor. Poster Presentation: Book of Abstracts; World Congress in Medical Physics/Bioengineering, San Antonio, Texas.

Malone, J.F., Maher, K.P., and O'Connor, M.K., 1988. Quantitative Aspects of Digital Fluoroscopy. Hospital Physicists' Association Conference Proceedings (in press).

Malone, J.F., Invited Paper at CEN/CENELEC Conference on Standardization of Medical Devices: "Radiation Protection-Ionizing Radiation".

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: BI6-F-135-UK

Head(s) of research team(s) [name(s) and address(es)]:

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Telephone number: 0235/83.16.00

Title of the research contract:

Evaluation of the radiation doses and risks associated with diagnostic X-ray examinations in Britain.

List of projects:

1. Assessment of the contribution of computed tomography to the collective dose from diagnostic radiology in Britain.

Title of the project no.:

Assessment of the contribution of computed tomography to the collective dose from diagnostic radiology in Britain.

Head(s) of project:

Mr B F Wall

Scientific staff:

Dr P C Shrimpton Dr D G Jones

I. Objectives of the project:

To obtain information on the pattern of use of CT scanners in Britain, on the number of patients undergoing each type of procedure and on the doses typically received by patients during those procedures.

II. Objectives for the reporting period:

1. To conduct survey measurements at the majority of the remaining 160 CT scanners in the UK.

2. To continue development of computational techniques to derive organ doses from measured axial dose profiles.

III. Progress achieved:

The continuation of the dose measurement survey at CT scanner facilities throughout the country has proceeded at a slower rate than originally anticipated. This is due to the diversion of staff and dosimetry resources to the European trial of the Quality Criteria for Diagnostic Radiographic Images being developed by this CEC contractors Study Group. The completion date for the CT survey contract has consequently been postponed by one year to 31 December 1989.

There are currently 207 CT scanners installed or on order in the UK comprising 42 different models from 11 different manufacturers. A sample of 75 scanners has been selected for inclusion in the dose measurement survey to provide a representative distribution of makes, models and geographical location.

By the end of 1988, free-in-air axial dose profile measurements were completed at one third of the scanners in the sample. For standard 8 or 10 mm slice thicknesses beam collimation was generally good with the full width-half maximum (FWHM) of measured dose profiles closely matching the nominal slice widths. However, for narrow (1 or 2 mm) slices most machines showed a deterioration in collimator performance with FWHM's four times the nominal slice width in the worst cases leading to a considerable build-up in patient dose when scanning contiguous slices. Values of the CT Dose Index (CTDI, defined as the integral dose along the axial direction of the single slice dose profile divided by the nominal slice width) for typical imaging conditions varied by a factor of 5 between different scanners; when normalised to the same mAs value they still varied by a factor of 3. Differences in beam filtration (which have been found to occur even between scanners of the same model) probably account for most of this variation.

Further Monte Carlo computer programs have been developed to simulate CT examinations with CRG and Siemens scanners. Conversion factors relating organ doses in a mathematical phantom to the measured axial CTDI have been calculated for series of contiguous scanned slices ranging from 0.5 cm to 8 cm thick. This allows the observation of the effect of calculated slice thickness on the accuracy of organ doses and

effective dose equivalents estimated for typical CT examinations. The effect on the conversion factors of displacing scanned volumes by up to ± 2 cm axially in the phantom has also been calculated so that the accuracy to which we need to know the exact position of CT scans within the patient can be estimated. A floppy disc containing a complete set of organ dose conversion factors for 200 contiguous 0.5 cm thick slices covering the head and trunk has been supplied to CEPN to facilitate the calculation of patient doses from the CGR scanners used exclusively in France. Similar data for a Siemens scanner are being compared with the results of Monte Carlo calculations from GSF, Munich as a check on the accuracy of the calculations.

Preliminary estimates of the effective dose equivalents for typical CT examinations on a CGR and Siemens scanner are shown in the table. Until the survey is complete it will not be known whether these values are representative for these types of scanner. The presence of complex shaped filters in the x-ray beam on many types of CT scanner has a major affect on patient doses and details of the construction of these filters are only just becoming available.

Examination	Effective Dose Equivalent (mSv)	
	CGR	Siemens
Head	1.5	4.0
Lungs	3.0	6.0
Abdomen	2.0	8.0

Arrangements have been made with the UK Institute of Physical Sciences in Medicine for the distribution of a questionnaire on CT practice to all operators of scanners in the UK. Details on patient workload, scanner servicing, physics support, quality assurance programmes, repeat rates and technique factors for up to 20 types of CT examination are required in the questionnaire. Local medical physicists will assist in their distribution and completion early in 1989.

IV. Objectives for the next reporting period:

To complete the national CT survey and report on individual and collective patient doses from current CT practice in the UK.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

D F Regulla, GSF, Munich, FRG

C Maccia, CEPN, Fontenay-aux-Roses, France

R Padovani, CRAD, Udine, Italy

E Vano, Complutense University, Madrid

VI. Publications:

No publications

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-136-I

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Telephone number: 0432/49.97.90

Title of the research contract:

Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure.

List of projects:

1. Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure.

Title of the project no.:

Refinement of methods for the assessment of organ doses, and possible reduction of patient exposure

Head(s) of project:

Dr. Renato Padovani
Servizio di Fisica Sanitaria
Ospedale S. Maria della Misericordia
Udine

Scientific staff:

Gilberto Contento
Maria Rosa Malisan
Mario Fabretto
Concettina Giovani

I. Objectives of the project:

To evaluate radiation risks in medical radiology and optimize protection in X-ray diagnostic procedures.
To assess frequencies of CT procedures in Italy and estimate collective doses.

II. Objectives for the reporting period:

1. To compare patient doses with those assessed in the 1983 survey in the same radiological departments.
2. To collect data on CT scanners operating in Italy and to perform measurements of CT doses in free air at the rotation axis in a representative sample of CT installations.

A. Urography (IVU) is one of the radiological procedures wich mostly contribute to collective doses. In the survey conducted in North-East Italy in 1983 this examination accounted for 11.8% of the total per caput-Effective Dose Equivalent (EDE) from radiological examinations. Since 1983 significant variations in the methods of conducting IVU have occurred so that a reappraisal of doses and frequencies was thought to be worth. The study was carried out in the major radiological institute of the region where 283 IVU examinations were analysed to assess which films are essential for reporting normal or pathological findings. Moreover, entrance skin dose for each exposure and breast and testis doses for the whole examination were measured in a sample of 16 patients with TLDs. Organ doses were evaluated by using NRPB Montecarlo conversion factors. As a cumulat' ' index EDE was used.
The mean number of ' ..res per examination has changed, from 1983 to 1988, from 11.5 to . and entrance skin dose for a full length view from 6.9 to 4.8 mGy. In table 1 are reported measured doses and evaluated EDE. The data collected showed that some films are particularly important for diagnosis and stressed the need of a flexible approach instead of a standard procedure as a first step to a dose reduction. Progresses in film-screen systems account for lower entrance skin dose while the increased use of non-ionizing techniques such as ultrasound is the preminent reason for reduced frequencies. Overall trends in IVU frequencies and doses are summarised in table 2.

Table 1. Mean dose received by patients for the main projections during IVU examinations (CV=% coefficient of variation).

Projection	Entrance skin mGy (CV)	Breast mGy (CV)	Testes mGy (CV)	EDE mGY (CV)
AP abdomen	4.83 (35)			0.56 (35)
Nephrotomogram	8.30 (27)	3.2 (112)	9.4 (127)	0.66 (27)
AP kidneys	4.68 (47)			0.28 (47)
AP bladder	4.86 (28)			0.38 (28)

Table 2. Trends of frequencies and doses for IVU.

Year	Frequencies exam/1000	EDE/examination mSv	per capita-EDE mSv/year	Reasons of changes
1978	16.8	not available		
1983	13	7.07	0.091	
1988	10.5	4.83	0.051	(*)
future	6.5	3.5	0.023	(**)

(*) Lower frequency due to ultrasounds; higher sensitivity of film-screen systems; less films/examination.
(**) Lower frequency due to ultrasounds; less nephrotomograms.

B. In the last years important interventional radiological procedures for the treatment of back pain have been developed. These procedures involve a rather large amount of irradiation for the patient and the evaluation of the Effective Dose Equivalent is of interest for a risk-benefit analysis of different therapeutic approaches.
In a sample of 25 patients in two neuroradiological italian departments, entrance skin, testis and breast doses were measured with TLDs and exposure area product with a Diamentor chamber. Organ doses were evaluated

by using NRPB Montecarlo conversion factors for the lateral view of lumbo-sacral joint (LSJ) projection.
The evaluated EDE was 0.5 mSv for chemonucleolysis treatment of lumbar disk herniation and 1.2 mSv for percutaneous discectomy by aspiration. Where comparing these results with available data concerning radiodiagnostic examinations, the two neuroradiological procedures are placed at a mid-level in the scale of EDEs. For the whole therapeutic procedure, which includes diagnostic examinations, interventional treatment and follow-up, the evaluated EDE was 14 mSv.

C. The questionnaire for the national CT survey has been forwarded to 297 radiological departments. So far, 70 questionnaires have been returned and filed into a computer database. Data concern technical features of CT scanners, programs of Quality Assurance and details on frequencies and procedural characteristics of examinations. In the meanwhile, the dosimetric survey started with the measurement of CT beam profiles at the rotation center in free air in a sample of CT installations. Profiles are determined by exposing across the beam a jig containing several lined-up LiF ribbons.
Preliminary results show that in Italy the installation rate of new CT scanners has grown up from 30 per year in the period 1982-86 to 50 per year in the last two years. The weekly workload of most installations ranges from 40 to 120 examinations performed during an active time of 40-70 hours. The relative frequencies of examinations are given in the following table:

EXAMINATION	FREQUENCY (%)
Head	40
Abdomen	18
Lumbar spine	14
Chest	10
Pelvis	7
Cervical spine	3
Petrous bone	2
Hypophysis	2
Limbs	2
Orbit	1
Other	1

Quality control tests are performed in almost all installations on a monthly basis. Tests are usually carried out by the manifacturer (75 cases out of 100).
Measurements on a sample of 10 scanners, irrespective of slice thickness, have yielded an average dose to air in free air at the centre of rotation of 0.98 mGy/mAs with a standard deviation of 0.27 mGy/mAs.

D. Patient doses in a selected group of X-ray examinations have been measured in 9 european X-ray departments which have actively participated to a trial organised by CEC aiming at the establishment of quality criteria for diagnostic radiographic images. Tha analysis of data showed no significant correlation between patient doses and image quality; however, the dose limits, provisionally suggested by the CEC protocol, were exceeded in 30% of the departments.

IV. Objectives for the next reporting period:

The dosimetric survey on a representative sample of italian CT installations will be completed. The data collected will be filed and analysed. Organ and collective doses for the italian population due to CT diagnostic activity will be evaluated.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

Carlo Maccia, CEPN, Paris, France
Barry Wall, Paul Shrimpton, NRPB, Chilton, UK
Ludovico Dalla Palma, Gino Gozzi, Roberto Pozzi Mucelli, F. Stacul, Istituto di Radiologia, Universita' di Trieste, Italy
Giuliano Fabris, Istituto di neuroradiologia, Ospedale Civile, Udine, Italy

VI. Publications:

G Contento, M R Malisan, R Padovani, C Maccia, B Wall, P Shrimpton, 'A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy, Br. J. Rad., 61,143-152, 1988.

L Dalla Palma, F Stacul, F Pozzi-Mucelli, G Contento, R Padovani, 'Urography: Optimization of the technique to lower patient exposure', Workshop:'Optimization of image and patient exposure in diagnostic radiology', Oxford, sept 1988.

C Maccia, B Wall, R Padovani, P Shrimpton, B Hussen, 'Resultas of a trial set up by a study group of the radiation protection programme of the CEC', Workshop:'Optimization of image and patient exposure in diagnostic radiology', Oxford, sept 1988.

G Contento, G Fabris, 'Dose al paziente nelle procedure neuroradiologiche per il trattamento dell'ernia discale lombare', to be published on Rivista di Neuroradiologia.

G Contento, M R Malisan, R Padovani, C Maccia, B Wall, P Shrimpton, 'A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy', Symposium: Radiation protection advances in Yugoslavia and Italy, June 1988, Udine, Italy.

C Maccia, R Padovani, 'Indagine europea sulla qualita' dell'immagine e la dose al paziente in radiodiagnostica: aspetti dosimetrici, metodologia e risultati preliminari', Convegno nazionale: Controlli di qualita' ed ottimizzazione nell'impiego delle radiazioni in medicina, AIFB, Dic 1988, Brescia, Italy.

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor:

Contract no.: BI6-F-137-D

Universität Erlangen-Nürnberg
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Head(s) of research team(s) [name(s) and address(es)]:

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Institut für Radiologie
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D-8520 Erlangen

Dr. Th. Schmidt
Radiologisches Zentrum Physik
Klinikum der Stadt Nürnberg
Flurstrasse 17
D-8500 Nürnberg 91

Telephone number: 09131/85-2310

Title of the research contract:

The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure.

List of projects:

1. The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure.

Title of the project no.:

BI6-F-137-D
The effective dose equivalent due to X-ray diagnostic examinations and the impact of quality control on medical exposure.

Head(s) of project:

Prof. Dr. rer.nat. Theodor Schmidt

Scientific staff:

Dr. rer.nat. H.-J. Rehm
cand.med. H. Erle
Dr. rer.nat. G. Böhnlein
Dr. rer.nat. M. Wucherer

I. Objectives of the project:

- Trial of devices for quality control in X-ray diagnostics
- Investigation of necessity and frequency of quality controls
- Effect of quality controls on radiation exposure
- Investigations about radiation exposure using new diagnostic methods (i.e. DSA)
- Determination of energy absorbed in typical X-ray investigations
- Determination of organ doses in a phantom and comparison with results of computer simulations (Drexler and co-workers)
- Determination of a malignancy-significant dose

II. Objectives for the reporting period:

- Investigation of long term constanty of dental X-ray units
- Dose reduction of lung examinations

III. Progress achieved:

1. Expediency of quality controls

a) Dental X-ray units

First controls of tube voltage, time of exposure, field size and radiation exposure carried out 1987 unmistakably proved considerable deviations between reported parameters and measured value (see project 1987 BI-6-0137-D). After the elimination of these defects, the parameters of the dental X-ray units have been traced for a few months. Quality controls have been performed weekly and showed that the values remain constant within a few percent. Typical results for the tube voltage of two different X-ray units are displayed in figure 1.

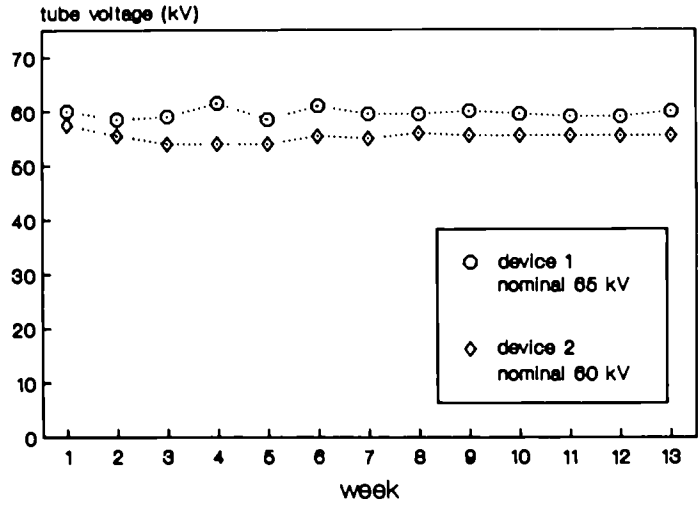

Figure 1: Tube voltage determined weekly for two different dental X-ray units.

As mentioned before in a different context, we think that "first pass" controls of X-ray units are reasonable and mostly reveal considerable

shortcomings. However, continuous controls in a short time seem unnecessary. Controls performed once in three months will be sufficiently frequent to ensure the radiation quality of X-ray units.

b) Digital subtraction angiography

Similar considerations hold for experiences with DSA X-ray units. Of course, the number of parameters of a DSA unit is substantially larger than that of a dental X-ray unit. The results of the radiation exposure measured during one year are shown in figure 2. The surprisingly large variation that occured once has been proved to be due to manipulations of service personnel on request of the physician (lack of image quality). Spontaneous variations have not been observed. The results of these measurements indicate, that constancy controls should be carried out whenever technical modifications or interventions have taken place.

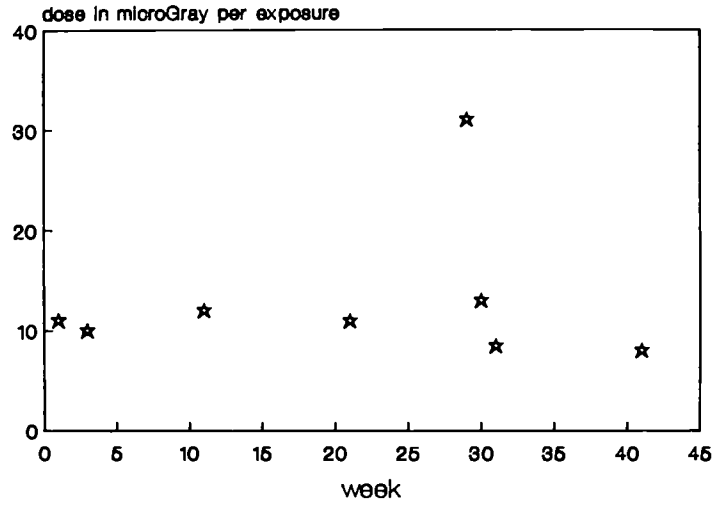

Figure 2: Radiation dose per exposure for DSA X-ray unit monitored occasionally for almost one year.

2. Reduction of radiation exposure of lung examinations

With 30 million exposures the lung examinations contribute primarily to the total number of X-ray exposures in the FRG. Although the radiation exposure of a single lung examination is small, the large number of examinations leads to a considerable contribution to the effective radiation dose of the population.

In order to reduce the radiation exposure, the industry developped two new methods, the so called "linear image intensifier" and the "large scale image intensifier". Basically, both systems work on the principle of the so called "indirect method". The dose reduction is achieved by electron-optical amplification and by the possible removal of an anti-scatter grid, respectively. The results of radiation exposure measurements of the new systems and of conventional systems are summarized in table 1. The radiation exposure of the conventional radioscopic screen photography exceeds the dose amount of the linear image intensifier by a factor of about 100.

Table 1: Radiation dose at an arbitrary reference point under identical conditions.

system	dose in mGy
radioscopic screen photography	6
direct radiography	0.6
large scale intensifier	0.1
linear intensifier	0.05

However, dose reduction must not be the only reason for the introduction of a new method. Objective quality criteria must be taken into account. Since the determination of the modulation transfer function for the different systems is difficult, resolution and contrast have been determined instead. In all cases the diagnostic quality has been sufficiently well for medical finding.

IV. Objectives for the next reporting period:

- Investigation of a system to quantify the image quality.
- Continuation with investigations considering the expedienca of quality controls.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

VI. Publications:

Schmidt Th. und H.-J. Rehm:
Ein neues Röntgenaufnahmesystem in der Lungenfürsorgestelle der Stadt Nürnberg
Röntgenstrahlen 57 (1987), 33-36

RADIATION PROTECTION PROGRAMME

Progress Report

1988

Contractor: Contract no.: BI6-F-214-E

Universidad Complutense
de Madrid
Ciudad Universitaria
Pabellon de Gobierno
E-28040 - Madrid

Head(s) of research team(s) [name(s) and address(es)]:

Prof. E. Vano Carruana
Catedra de Fisica Médica
Facultad de Medicin
Universidad Complutense
E-28040 - Madrid

Telephone number: 243.18.61

Title of the research contract:

Optimization of Protection in Medical Diagnostic Radiology.

List of projects:

1. Optimization of Protection in Medical Diagnostic Radiology.

Title of the project no.:

OPTIMIZATION OF PROTECTION IN DIAGNOSTIC RADIOLOGY

Head(s) of project:

Prof. E. Vañó and Prof. L. González

Scientific staff:

Profs. A. Calzado, V. Delgado, P. Morán, P. Ortiz y M. Marín.

I. Objectives of the project:

1 - Analysis of risks from Diagnostic Radiology to operating staff and population, through area and personal dosimetry, and its correlation with equipment features, workloads and skills and training of operating staff.

2 - Analysis of risks to patients, by means of direct dose measurements and numerical estimates.

3 - Scheming and putting into practice of a pilot plan of Quality Assurance in Diagnostic Radiology at a hospital level.

4 - To set up the bases for a nation-wide future programme of population collective dose estimate from Diagnostic Radiology.

II. Objectives for the reporting period:

1 - Conclusions on the risk analysis to radiological staff through personal and area dosimetry.

2 - Analysis of the working technical conditions and protocols of the radiological examinations bearing a higher contribution to the collective effective dose equivalent.

3 - Patient dose estimates using TLD, transmission ion chambers and numerical approaches.

III. Progress achieved:

Methodology.-

Personal dosimetry to staff has been sometimes carried out by duplicating (for comparison purposes) the dosemeters from which whole body doses were estimated, apart from using additional dosimeters for special risk organs (hand and lens) when relevant. Part of these results have been stored in a computer, with the aim of correlating them with the number and type of examinations performed, radiologic equipment used, room performances and training and skills of staff.

Area dosimetry has been performed with TLD LiF chips, collecting data from between 5 and 10 measurement points per room along periods between 2 and 8 months, recording and computing at the same time the number and type of the examinations.

Referring patient dosimetry, special care has been paid so as to obtain practical data picturing the current situation existing in the centres before the beginning of the QA plan. The most significative parameters of each examination (exposure number, screening time, kVp, mAs, focus to skin distance, field size etc) likewise equipment features (total filtration, rectification, film-screen combination, processing facility, etc) and the operator name. Estimates have been performed at three levels:

a) Measuring entrance dose with ionization chambers, simulating typical conditions of each examination in each room, previously determined over a sufficiently significant patient sample. This level was occasionally applied in simple examinations such as chest, spine, etc., and on samples showing low dispersion.

b) Measuring individual entrance dose (and some organ doses when possible) on a sample of patients with thermoluminiscent (TL) dosemeters or a Diamentor large area transmission chamber (PTW-Freiburg, Germany). This was the most usual practice.

c) Measuring organ doses in REMAB and RANDO phantoms (Alderson, USA), in complex examinations (GI tract, urography, CT, angiography, etc.) using contrast media if necessary, and with a total of 70-100 TL dosemeters per simulation, aside from occasional simultaneous measurement of the (dose x area) product, in order to apply results later on, with approximations and corrections to measurements actually performed on patients.

Organ doses were mainly derived, whenever possible, using the coefficients calculated by Jones and Wall from a mathematical phantom and Monte Carlo methods. Suitable approaches (deduced from direct measurements with phantoms) in the case of complex examinations have been applied.

Results.-

Area dosimetry values show that doses at the diagnostic radiology installation environtments are negligible. A better signposting within the services can avoid accidental exposures. Only 10% of the analysed rooms had correct warning signals and access control.

With reference to operation staff, the detected anomalous values (i. e., those over 3/10 the annual effective dose equivalent limit) have been attributed to design deficiencies in structural shielding, unsuitable operation procedures (screening times too large and irregular using of the leaded apron) and obsolete equipment (intensifiers without TV monitor, for instance). Work load has been a dominant parameter in catheterization and traumatology surgery rooms. Resident radiologists, with a lower Radiation Protection training, have received slightly higher doses than the remainding personnel. Notwithstanding, this abnormal findings affect to less than 5% of the total of operators controled (about 200 people).

Concerning patient dose monitoring, special emphasis has been given to the examinations used by the CEC working group in the work relating image quality and doses, likewise to gastro-intestinal tract, digital angiography and CT examinations. Data reported here have been obtained before implementing QC measures.

IVU examinations show a number of images per examination ranging from 4.7 to 8.8, depending on the protocol of each room. The entrance dose value, averaged over more than 1 600 examinations becomes 12 mGy, 20% over the provisional maximum acceptable value (PMAV) suggested by the CEC.

In the case of barium enema, very different diagnostic contents seems to be obtained in the rooms monitored, that give rise to variations in the image numbers (between 6.3 and 14.3). A weighted mean effective dose equivalent per digestive tract examination value of 9.7 mSv is obtained for the Madrid area, from Diamentor lectures togheter with organ TLD in phantoms.

Some other results before QC show weighted mean values for the entrance dose up to five-fold the PMAVs, as an outcome of a number of already well established causes, some of them immediately successfully corrected. Mean effective dose equivalent values (in mSv) obtained in the Madrid area have been: 0.29 for chest; 0.14 for skull; 2.1 for spine; 2.0 for hip and pelvis; 1.2 for abdomen and 6.6 for IVU, among others.

Contributions to the genetically significant dose have been evaluated for some of the selected examinations, obtaining 43.5 μGy for abdomen, 39.1 μGy for lumbar spine, 44.0 μGy for hip and pelvis, 67.3 μGy for IVU and 0.28 μGy for chest.

IV. Objectives for the next reporting period:

1) Final results of the patient dosimetry.

2) Design and first results of the QA pilot programme.

3) Design of a nation-wide programme for patient dose evaluation.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]:

- San Carlos, Doce de Octubre y Gómez Ulla University Hospitals, La Princesa Hospital and Hermanos Miralles Outpatient Centre (Diagnostic Radiology and Radiation Protection Units).
- Dosimetry Unit, Department of Health and Consumer Affairs.
- Energy Study Institute from CIEMAT.
- B. F. Wall, NRPB, United Kingdom.
- C. Maccia, CEPN, France.

VI. Publications:

1) Proyecto sobre evaluación de riesgos y control de calidad en instalaciones de radiodiagnóstico. I.-Planteamiento del proyecto. L. González y E. Vañó.
Radiología, 30(5), 273-278 (1988) (in Spanish).

2) Proyecto sobre evaluación de riesgos y control de calidad en instalaciones de radiodiagnóstico. Medidas y evaluaciones dosimétricas en estudios radiológicos de tórax. L. González, E. Vañó, M.J. Ruiz, P. Fernández Letón, M.J. Manzanas y M.L. Marco. In press in "Radiología" (in Spanish).

3) Some indicative parameters on diagnostic radiology in Spain. First dose estimations. E. Vañó, L. González, A. Calzado, P. Morán and V. Delgado. Brit. J. Rad. 62, 20-26 (1989).

4) ALARA and Radiodiagnostics. Third European Scientific Seminar on "Radiation Protection Optimization". Madrid, September, 12 to 15, 1988. E. Vañó, L. González, C. Maccia and B. F. Wall. 26 pp. In press in the Seminar Proceedings.

IV

KOORDINIERUNGSTATIGKEIT

COORDINATION ACTIVITIES

ACTIVITES DE COORDINATION

IV. Coordination

Study Group meetings, workshops, seminars and symposia have proved to be a most effective means of coordination because they are naturally adapted to scientific work and easily accepted by scientists. These meetings, focussing on the evaluation of particular subject areas of the Radiation Protection Programme, are attended by research workers involved in the contract programme, as well as scientists from non-participating laboratories or organizations and scientific staff members of the Commission.

On the following pages the various meetings held in 1988 are listed:

A: Meetings of Study Groups, where scientists involved in the contract programme, independent experts and staff members of the Commission discuss specific subject areas of the programme.

B: Meetings organized or co-organized by the Commission of the European Communities on special subject areas of interest for radiation protection and where contacts among scientists from a wider range of disciplines and countries might be established.

C: Meetings of experts appointed for the purpose of coordinating and stimulating efforts towards practical measures of radiation protection as foreseen in Chapter III of the EURATOM Treaty or convened by the Commission for special tasks.

A

MEETINGS OF STUDY GROUPS IN 1988

Study Group "Co-ordination MARIA programme"

Chilton (GB), 16-17 January 1988
15 participants from 2 countries and the Commission

Principal subjects :
- Outline of the MARIA code
- Structure of the code
- File interface formats

Study Group "Improvement of reliable long-distance atmospheric transport models"

Pavia (I), 18 January 1988
10 participants from 4 countries and the Commission

Principal subjects :
- Data analysis
- Complex terrain modelling
- Source term estimation
- Emergency response

Study Group "Underlying data for derived emergency levels", Post-Chernobyl action

Neuherberg (D), 10-11 February 1988
18 participants from 4 countries and the Commission

Principal subjects:
- Radioecological models for the assessment of internal doses
- Distribution and comsumption of food
- Metabolic and dosimetric models for the assessment of internal doses
- Measurement of the internal contamination of man
- Emergency management

Study Group "Co-ordination : Countermeasures in the rural environment"

Bilthoven (NL), 22-23 February 1988
7 participants from 4 countries and the Commission

Principal subjects :
- Removal of contamination of agricultural products
- Food processing as a decontamination technique
- Agro-technical aspects of decontamination
- Preparation of the first joint report.

Study Group "Co-ordination : Evaluation of data on the transfer of radionuclides in the foodchain"

Rome (I), 22 March 1988
9 participants from 7 countries and the Commission

Principal subjects :
- Impact of chemical speciation on the radionuclide transfer in terrestrial ecosystems
- Contamination of natural ecosystems
- Tranfer to animals and animal products
- Contamination of aquatic ecosystems
- Preparation of the first joint report

Study Group "RESSAC programme"

Cadarache (F), 17 May 1988
7 participants from 2 countries and the Commission

Principal subjects :
- Technical aspects of the countermeasure programme
- Possible benefits for potential participants

Study Group "Improvement of reliable long-distance atmospheric transport models"

Athens (GR), 2-3 June 1988
10 participants from 4 countries and the Commission

Principal subjects :
- Source term estimation
- Data analysis
- Emergency response
- Complex terrain modelling
- Preparation of a joint report

Study Group "Monitoring and surveillance in accident situations", Post-Chernobyl action

Brussels (B), 3 June 1988
9 participants from 7 countries and the Commission

Principal subjects :
- Information of progress and state of individual projects and future plans
- Coordination of research activities for field measurements of radioactivity

Study Group "Co-ordination : Evaluation of data on the transfer of radionuclides in the foodchain"

Brussels (B), 12 July 1988
16 participants from 7 countries and the Commission

Principal subjects :
- Transfer in terrestrial ecosystem
- Contamination of natural ecosystems
- Contamination of aquatic ecosystems
- Validation of soil-plant parameters
- Preparation of the final report

Study Group "Steering Committee of the Thorotrast Programme"

Heidelberg (D), 2 September 1988
25 participants from 3 countries and the Commission

Principal subjects :
- Most recent epidemiological data in thorotrast patients
- Ongoing animal experiments and future plans
- Preparation of Radium-Thorotrast workshop

Study Group "Co-ordination : Countermeasures in the rural environment"

Bilthoven (NL), 15 September 1988
12 participants from 6 countries and the Commission

Principal subjects :
- Agro-technical aspects of decontamination
- Removal of contamination of agricultural products
- Effectiveness and practicability of rural countermeasures
- Preparation of the final report.

Study Group "Quality assurance in diagnostic radiology"

Oxford (GB), 30 September 1988
16 participants from 10 countries and the Commission

Principal subjects :
- Review of the intercomparison programme in diagnostic radiology
- Elaboration and discussion of guidelines for dosimetry in diagnostic radiology
- Future intercomparison action

Study Group "Underlying data for derived emergency levels"

Paris (F), 23-24 October 1988
14 participants from 4 countries and the Commission

Principal subjects :
- Evaluation of the hypothesis underlying the Article 31 methodology
- Radioecological models for the assessment of internal doses
- Distribution and consumption of food
- Metabolic and dosimetric models
- Emergency management

Study Group "Restricted intercomparison programme on environmental dosemeters"

Luxembourg (L), 26-27 October 1988
6 participants from 4 countries and the Commission

Principal subjects :
- Preparation of the part II of the report of intercomparison programme of environmental gamma doserate meters
- Future work

Study Group "Quality criteria for diagnostic radiographic images"

Brussels (B), 15-16 November 1988
7 participants from 3 countries and the Commission

Principal subjects:
- Establishment of the revised list of quality criteria
- Evaluation of quality criteria for paediatric radiological examinations

Study Group "Improvement of realistic countermeasures in the urban environment"

Glasgow (GB), 17-18 November 1988
8 participants from 3 countries and the Commission

Principal subjects:
- Chemistry of retention on building materials
- Results of the decontamination experiments in Sweden
- Preparation of the joint progress report

Study Group "Steering Committee Pathogenesis of somatic radiation hazards"

Munich (D), 21 November 1988
15 participants from 2 countries and the Commission

Principal subjects :
- Data from animal experiments studying the effects of dose and dose rates on osteosarcoma.
- Updating of cancer data in the low and high dose group of radium patients.
- Radium-induced cataract and its dose-effect relationship.
- Recent risk assessment based on the Japanese survivor data.

Study Group "Co-ordination : Behaviour of Tritium in the environment"

Hannover (D), 13-14 December 1988
6 participants from 3 countries and the Commission

Principal subjects :
- Results of the tritium release experiments in Canada and France
- Evaluation of the importance of the release of volatile organic tritiated compounds.

B

MEETINGS ORGANIZED OR CO-ORGANIZED BY

THE COMMISSION OF THE EUROPEAN COMMUNITIES IN 1988

EULEP Task Group Meeting on "Cellular basis of late vascular changes in irradiated brain"

Mol (B), 20-21 January 1988
7 participants from 4 countries

Principal subjects:
- Delineation of areas at risk in the irradiated brain
- Identification of earliest pathophysiological and pathological change
- Correlation with circulatory physiology

Workshop on "Recent advances in reactor accident consequence assessment"
Co-organized with OECD-NEA (Organization of Economic Cooperation and Development - Nuclear Energy Agency), Paris and ENEA (Comitato Nazionale per la Ricercha e per le Sviluppo dell'Energia Nucleare e delle Energie Alternative), Rome

Rome (I), 25-29 January 1988
120 participants from 13 countries and the Commission

Principal subjects:
- General accident consequence assessment code development
- Atmospheric dispersion
- Exposure pathways
- Health effect models
- Countermeasures and economic consequences
- Uncertainty analysis
- Applications of accident consequence assessment

EULEP Task Group Meeting on "Cell biology of lymphomas"

Brussels (B), 28-29 January 1988

15 participants from 2 countries

Principal subjects:
- Thymic grafts to thymectomised, irradiated animals
- Transfer of preleukaemic cells to irradiated recipients
  Cytogenetic studies on thymic lymphoma
  Lymphokines and growth factors
  Receptors for neurotransmitters

Workshop on "Technical and physical parameters for quality assurance in medical diagnostic radiology : tolerances, limiting values and appropriate measuring methods"

Brussels (B), 23-25 February 1988
120 participants from 18 countries and the Commission

Principal subjects:
- Requirements on measuring methods, instrumentation and techniques for quality control
- Limiting values for certain technical and physical parameters
- Effects on dose reduction
- Effects on image quality
- Possibilities for standardizing tolerances of measuring methods and limiting values for quality control parameters

EURADOS Working Committee 1 : "Development and implementation of microdosimetric instruments and methods for radiation protection"

Braunschweig (D), 1-2 March 1988
15 participants from 5 countries

Principal subject :
- Interpretation and publication of results of the second intercomparison of dose equivalent meters based on microdosimetric techniques

EULEP General Assembly

Reisensburg (D), 7-10 March 1988
70 participants from 8 countries

Principal subjects :
- Presentation of EULEP activities in 1987.
- Proposals for task groups.
- Presentation and discussion of future EULEP activities.
- Task group and standardization group meetings

Workshop on "Biological assessment of occupational exposure to actinides"
Co-organized with the CEA (Commissariat à l'Energie Atomique), Fontenay-aux-Roses

Versailles (F), 30 May - 2 June 1988
101 participants from 17 countries and the Commission

Principal subjects :
- Potential sources of exposure to actinides.
- Review of toxicity of uranium, plutonium and other actinides.
- Assessment of exposure to uranium, plutonium and other actinides.
- Development of models for determining and predicting metabolic behaviour after human exposure.
- Determination of metabolic characteristics of actinides.
- Management of overexposure.

EURADOS-CENDOS Committee-2 : "Skin dosimetry and surface contamination monitoring"

Braunschweig (D), 10 June 1988
14 participants from 5 countries

Principal subjects :
- Biological effects. ICRP Task Group
- Computer programmes and results :
  - Absorbed dose from external beta- and low-energy photon radiations
  - Absorbed dose from contamination of the skin
  - Hot-particle project
  - Benchmark experiment, $^{90}Y/^{90}Sr$ source
- The extend of weakly penetrating radiation at the workplace
- Personal dosimetry
- Survey dose rate meters
- Surface contamination monitoring
- Measurements of dose rates from non-standard beta-ray sources

EURADOS /CENDOS Working Committee 5, Sub-Committee : "Track Etch"

Karlsruhe (D), 14-15 June 1988
10 participants from 5 countries

Principal subjects :
- Plastic manufacture and characteristics
- Report of International track-etch meeting
- Background survey
- Joint irradiation programme
- Interface with radon group

CEC - AECL (Atomic Energy of Canada Ltd) - Coordination Meeting

Chalk River (Canada), 18 June 1988
20 participants from Canada and the Commission

Principal subjects:
- Presentation of the Radiation Protection Programme of the CEC
- Presentation of the research areas of Atomic Energy of Canada Ltd, Health Sciences
- Major problems in radiation biology, epidemiology, tritium R.B.E., screening assays, in vitro recombination techniques, cancer risk modification
- Objectives of the environmental science and technology group, studying the transfer of radionuclides
- Common points of interests between both research programmes

CEC-DOE (Department of Energy) Co-ordination Meeting

Annapolis, MD (USA), 20-22 June 1988
24 participants from the U.S.A. and the Commission

Principal subjects:
- Presentation of research areas where cooperation exists between CEC and DOE
  - Mammalian radiology
  - Cellular and molecular radiology
  - Microdosimetry
  - Radon research
  - Atmospheric modeling
- Presentation of the work of CIRRPC (Committee on International Radiation Research and Policy Coordination)
- Overview of work of the DOE Environmental Measurement Laboratory
- Present state of development of the human genome project
- The DOE subsurface transport programme
- Further coordinated actions

Workshop on "Critical evaluation of radiobiological data for biophysical modelling"
Co-organized with the US-DOE (Department of Energy)Washington DC

Oak Ridge (USA), 22-25 June 1988
32 participants from 5 countries and the Commission

Principal subjects :
- Cellular and molecular data
- Animal data
- Physical and chemical data

EURADOS - Subcommittee 5 : "Characteristics of ionization chambers"

Rijswijk (NL), 25-26 August 1988
6 participants from 3 countries

Principal subject :
- Joint experiments on the relative neutron sensitivity of argon filled magnesium chambers over the energy range from 5 to 19 MeV.

Workshop on "Transfer of radionuclides to livestock"

Co-organized with the NRPB (National Radiological Protection Board) Chilton

Oxford (GB), 5-8 September 1988
60 participants from 16 countries and the Commission

Principal subjects :
- Transfer of radionuclides to animals - an historical perspective
- Retention charachteristics of radionuclides in pasture and other animal feed
- Uptake of radionuclides by animals - effects of environmental and farming practices
- Animal metabolism - absorption from the gut, effect of chemical form, distribution and retention, effect of age and species
- Development of predictive models and model validation
- Measures to prevent uptake of radionuclides

Workshop on "Methods for determining differential radiosensitivity in humans"
Co-organized with the AECL (Atomic Energy of Canada Limited) Chalk River

Brighton (GB), 7-9 September 1988
24 participants from 6 countries and the Commission

Principal subjects :
- Fibroblast and lymphocyte survival
- Chromosome assays
- Effects on nucleoids and DNA complexes
- Other biochemical approaches

Symposium on "Physical, Biological and clinical Aspects of Total Body Irradiation (TBI)" jointly organized by EULEP, ESTRO (European Society for Therapeutic Radiation Oncology) and EBMT (European Marrow Bone Transplant Group)

The Hague (NL), 7-9 September 1988
130 participants from 21 countries

Principal subjects :
- Techniques and dosimetry for total body irradiation
- Clinical basis for total body irradiation fractionation
- Immunohaematological aspects of total body irradiation
- Late effects after bone marrow transplantation

Third European Scientific Seminar : "Radiation protection optimization"

Madrid (E), 12-14 September 1988
150 participants from 16 countries and the Commission

Principal subject :
- Progress made in practical applications of the optimization principle in the domain of design and operation of nuclear and industrial facilities, natural radiation, medical practices, countermeasures.

Seminar on "Applications, perspectives and limitations of comparative risk assessment and risk management"
Co-organized with CEDHYS (Centre de Developement des Etudes et Applications en Hygiene et Sécurité) Paris

Nice (F), 26-30 September 1988
100 participants from 21 countries and the Commission

Principal subjects :
- Aims of comparative risk assessment
- Methods and their assumptions
- Case studies at different levels
- Limits of validity
- Risk management strategies
- Risk perception
- Future perspectives

Workshop on "Optimization of image quality and patient exposure in diagnostic radiology"
Co-organized with the NRPB (National Radiological Protection Board) Chilton

Oxford (GB), 27-29 Septembre 1988
150 participants from 19 countries and the Commission

Principal subjects :
- Image perception
- Physical assessment of image quality
- Clinical assessment of image quality
- Optimization of specific imaging processes
- Patient dose measurements and quality assurance
- Quality criteria for diagnostic radiographic images

EULEP Task Group Meeting on "Molecular approach to the study of radiation-induced osteosarcoma and lymphoma"

Bordeaux (F). 29-30 September 1988
17 participants from 5 countries

Principal subjects:
- Participation of retroviruses
- Specific chromosomal aberrations as an early event in leukaemogenesis
- Role of oncogenes
- Search for bone-specific differentiation genes
- Role of cytokines
- Gene amplification in vitro; novel cloning vectors

Workshop on "Risks from radium and thorotrast"
Co-organized with the US-DOE (Department of Energy), Washington DC and the US National Cancer Institute, Bethesda

Bethesda, MD (USA), 3-5 October 1988
30 participants from 9 countries and the Commission

Principal subjects :
- Cancer due to Ra-224 and Ra-226.
- Non-stochastic damage due to Ra-224 and Ra-226.
- Animal data on Ra and related radionuclides.
- Epidemiology of thorotrast
- Risk assessment based on studies of effects of radium and thorotrast.

EURADOS-CENDOS Working Committee 6 : "Assessment of internal dose"

Frankfurt (D), 4-5 October 1988
8 participants from 3 countries

Principal subjects :
- Project of research proposal on stable isotopes
- Project of a registry of radiation workers exposed to internally deposited radioactivity, including autopsy data
- Intercomparisons of methodologies for internal dose assessments extension from UK to a European Intercomparison
- Collaboration with EULEP
- Internal dosimetry

EURADOS Working Committee 4 : "Dissemination and development of computer programmes for dosimetric problems (Numerical Dosimetry)"

Cadarache (F), 13-14 October 1988
12 participants from 3 countries

Principal subjects :
- Application of unfolding procedures for Bonner spheres
- Calculations of effective and organ doses due to Cs contamination of the soil after the Chernobyl accident.

Seminar "MARIA and UFOMOD (Untallfolgenmodelle)"
Co-organized with KfK (Kernforschungszentrum), Karlsruhe

Karlsruhe (D), 17-21 October 1988
50 participants from 10 countries and the Commission

Principal subjects:
- Teach in for potential users of the UFOMOD code
- Optimization of the documentation of the COSYMA code
- Progress within the MARIA programme

Workshop on "Implementation of dose-equivalent meters based on microdosimetric techniques in radiation protection"
Co-organized with the GSF (Gesellschaft für Strahlen- und Umweltfoschung), Neuherberg

Schloss Elmau (D), 18-20 October 1988
48 participants from 10 countries

Principal subject :
- **Application of low pressure proportional counters and related instruments in radiation protection**

EURADOS Working Committee I : "Development and implementation of microdosimetric intruments and methods for radiation protection"

Schloss Elmau (D), 20 October 1988
11 participants from 4 countries

Principal subjects :
- **Results of international TEPC (Tissue Equivalent Proportional Counter) intercomparison**
- **Future research needs for microdosimetric application in practical radiation protection**

EULEP Workshop on "Neoplastic and non-neoplastic lesions of haemopoietic tissues" organised by the EULEP Committee of Pathology

Munich (D), 4-5 November 1988
25 participants from 7 countries

Principal subjects:
- Applications of immunohistochemical methods
- Total lymphoid irradiation
- Late effects of irradiation on the bone marrow of dogs
- Molecular biological aspects of follicular lymphomas in mice
- Tumour heterogeneity in follicular lymphomas in man
- Transplantable multiple myeloma in mice

EULEP Task Group Meetings on "Stem cells, bone-seeking radionuclides, and fetal dosimetry"

Chilton (UK), 7-9 November 1988

29 participants from 5 countries

Principal subjects:
- Short- and long-term response of mouse bone marrow to plutonium-239 and radium-224; effects on stem cells and on stromal tissue
- Lymphoma and leukaemia induction in mice from low levels of radium-224
- Alpha-dose to bone marrow from environmental levels of radionuclides
- Transfer of actinides to the fetus and newborn animal, distribution in the fetus and some late effects
- Dosimetric models for the fetus
- Effects of external in internal irradiation on the developing brain

EULEP Task Group Meeting on "Interspecies comparsion of lung clearance"

Chilton (UK), 9-10 November 1988
17 participants from 4 countries

Principal subjects:
- Continuation of interspecies clearance studies with "porous" and "solid" cobalt oxide particles
- Interspecies clearance studies of ionic cobalt instilled into the lung
- Redistribution of particles in lung tissue
- Intraphagolysosomal pH in alveolar macrophages and rate of dissolution of particles
- Functional studies and early radiation damage in alveolar macrophages

EURADOS-CENDOS Committee-2, Working Group 6 : "The assessment of skin dose rates from particulate beta-ray sources"

Grenoble (F), 24 November 1988
4 participants from 2 countries

Principal subject :
- Working programme for measuring and calculating dose rates from a range of ^{60}Co particulates of various sizes.

EURADOS - CENDOS Council Meeting

Paris (F), 24 November 1988
12 participants from 5 countries and the Commission

Principal subjects :
- Project of establishing a working party on electronic dosemeters
- Project of a symposium on general radiation dosimetry

International Seminar : "Radiation protection training and information for workers"

Luxembourg (L), 28-30 November 1988
100 participants from 14 countries and the Commission

Principal subjects :
- Identification and analysis of needs of various levels
- Different national approaches and the institutions concerned
- Training models
- Courses and syllabuses
- Educational aids
- Information transmission policies and the monitoring of their impact

The participation of workers in training and information

EULEP Task Group Meeting on "Decorporation treatment after contamination with actinides"

Karlsruhe (D), 1 December 1988
6 participants from 4 countries

Principal subjects:
- Status experiments with pure LICAM(C)
- Progress on collaboration with University of California
- Possible new chelating agents: preliminary trials in vitro
- Progress in other decorporation studies in EULEP laboratories

International Seminar for the intention of the representatives of the "International Society of Radiographers and Radiological Technicians"

Luxembourg (L), 5-7 December 1988
40 participants from 16 countries and the Commission

Principal subject :
- Uniform application of the Council Directive of 3 September 1984 laying down basic measures for the radiation protection of persons undergoing examination or treatment

International Seminar "Information about radiation protection" for the representatives of the European Trade Union confederation

Luxembourg (L), 12-13 December 1988
20 participants from 12 countries and the Commission

Principal subject :
- Problems concerning the protection of occasionnaly exposed workers in nuclear installations against the dangers of ionizing radiation and a proposal for a Council Directive on informing the population about health protection measures in the event of a radiological emergency

EURADOS - Subcommittee 5 : "Characteristics of ionization chambers"

Teddington (GB), 15-16 December 1988
4 participants from 3 countries

Principal subject :
- Joint experiments on the relative neutron sensitivity of argon filled magnesium chambers over the energy range from 5 to 19 MeV.

C

MEETINGS OF EXPERTS IN 1988

Group of Experts referred to in Article 37 of the Euratom Treaty

Brussels (B), 14-15 March 1988
40 participants from 12 Member States

Principal subject :
- Neckar II (D) and Vandellios II (E) nuclear power stations

Group of Experts referred to in Article 31 of the Euratom Treaty

Luxembourg (L), 17-18 March 1988
29 participants from 12 countries and the Commission

Principal subjects :
- Draft directive on the supervision and control of the transfrontier shipment of radioactive waste
- Maximum permitted radioactivity levels in animal feedingstuffs in the event of a nuclear accident
- Maximum permitted radioactivity levels for liquid foodstuffs and for baby food
- Draft recommendation for the protection of the public against radon in dwellings

Group of Experts referred to in Article 31 of the Euratom Treaty

Brussels (B), 24 October 1988
29 participants from 12 countries and the Commission

Principal subjects :
- Draft directive on the supervision and control of the transfrontier shipment of radioactive waste
- Maximum permitted radioactivity levels in animal feedingstuffs in the event of a nuclear accident
- Maximum permitted radioactivity levels for liquid foodstuffs and for baby food
- Draft recommendation for the protection of the public against radon in dwellings

Group of Experts referred to in Article 37 of the Euratom Treaty

Luxembourg (L) 7 November 1988
40 participants from 12 Member States

Principal subject :
- Trillo (E) nuclear power station

V

AUSWAHL EINIGER AUF VERANLASSUNG DER KOMMISSION ERSCHIENENER VEROFFENTLICHUNGEN

SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE OF THE COMMISSION

CHOIX DE PUBLICATIONS EDITEES A L'INITIATIVE DE LA COMMISSION

V. Publications 1988

The scientific research results of the Commission's Radiation Protection Programme are presented in articles published in scientific journals. References to these are given in the corresponding Progress Reports. In certain cases the Commission initiated surveys of detailed results of specific activities in the field of radiation protection and published them as monographs, proceedings and data collections. Short descriptions of those publications, prepared in 1988, are given on the following pages.

Radiation Protection in the European Community - Evaluation and Suggestions

Report established by a Committee of high level independent scientists G. BENGTSSON, W. JACOBI, H. JAMMET, E. POCHIN, G. SILINI, A. WAMBERSIE

The accident at Chernobyl has highlighted the need for an independent assessment of the present situation and future actions with respect to the protection of the population in the European Community from the danger of ionizing radiation.

The Commission of European Communities has therefore convoked a "Committee of high-level independent scientists", asking them :

- **to assess the scientific evidence arising from the current research in view of recent nuclear incidents and to consider the possible implimentations for the Basic Standards and emergency reference levels; and**
- **to advise the Commission on future actions in radiological protection.**

The Committee met four times between July and November 1986 and discussed all aspects dealing with radiation protection in routine operations and after accidents. The Committee considered the adequacy of present safety standards in the light of available scientific knowledge and paid special attention to radiation protection following nuclear incidents. Rationales for different countermeasures were reviewed and, in particular, the different aspects and methodologies for defining derived emergency reference levels for radioactive contamination in foodstuff were analysed.
The Report has been written in English. The translations of chapter "Summary conclusions and recommendations" into all official languages of the Commission are added as appendices to the Report.

Report EUR 11449 EN, 1988, 283 pages

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price : ECU 21

The impact of conventional and nuclear industries on the population: A comparative study of the radioactive and chemical aspects

Edited by R. COULON, J. AIGUEPERSE and F. ANGUENOT

This study was carried out to make it possible to assess and localize, in an objective manner, the extent of the hazards and associated detrimental effects which are inherent in nuclear and non-nuclear industrial activities, among all the hazards to which the population of a given region is exposed. Rather than carry out a purely theoretical and speculative study, the case of a region as a basis, was chosen to carry out a full-scale exercise, taking into account the existing real situation. The region chosen is situated in the south-east of France (Greater Rhône Delta) where almost all industrial activities can be found: electricity generating industries (thermal and nuclear power stations), the activities associated with them (extraction, processing, storage of waste, ...). To put the risks of all these activities to workers, public and environment in perspective, the case of other sources of risk such as certain agricultural and medical activities, as well as exposure to natural radiation, were considered. The methodology developed from this study should be useful for authorities called upon to define economic orientations, industrial development and pollution abatement programmes on a regional scale, by providing an objective view of the hierarchy of risks and their distribution within the population.

Report EUR 10557, 1988; 343 pages; 52 figures; 55 tables

To be ordered through:

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 30

Intercomparison of environmental gamma dose rate meters

A comprehensive study of calibration methods and fiels measurements.

The measurements of ambient radiation in the neighbourhood of a nuclear installation is essential to demonstrate that members of the public are not exposed to unacceptable levels of radiation arising from the operation of the installation.

The Commission of European Communities decided during 1983 to help Member States to check the performance of their monitors at background radiation levels and to investigate different calibration techniques. This report describes the results of the intercomparison programme of environmental gamma dose rate meters organized during 1984 and 1985.

Details are also given of the evaluation of ambient background radiation at locations where the relative contributions to the air kerma rates from cosmic and terrestrial radiations were significantly different.

Radiation Protection Series N° 41

Report EUR 11665 EN, 120 pages, in press

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Investigation of radiation protection instruments based on tissue-equivalent proportional counters, Result of a EURADOS intercomparison

Edited by G. DIETZE, A.A. EDWARDS, S. GULDBAKKE, H. KLUGE, J.B. LEROUX, L. LINDBORG, H.G. MENZEL, V.D. NGUYEN, Th. SCHMITZ, H. SCHUHMACHER

Different prototypes of dose equivalent meter for area monitoring, which have been developed on the basis of tissue-equivalent proportional counters by several groups in Europe during the past several years, were intercompared in various reference neutron and photon fields of the PTB in Braunschweig. The instruments are able simultaneously to measure ambient dose equivalent due to neutrons and photons and to determine a mean quality factor of the radiation. The energy dependence of the response to neutrons was investigated for neutron energies between 73 keV and 5MeV. While the responses of the systems generally decrease with decreasing neutron energy, significant differences are also observed. These differences can be attributed to variations in the detector wall thickness and the simulated cavity diameters. The results are further discussed with respect to the detector calibration procedures used and the uncertainty of measurements caused by statistical fluctuations of the count rates and the lineal energy distributions.

Report EUR 11867 EN, 1988, 36 pages

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price : ECU 5

Report on the results of the third intercomparison study of thermoluminescent dosemeters for environmental measurements

Edited by C.M.H. DRISCOLL

Intercomparison exercises of thermoluminescence detectors generally involve sending dosemeters to a central organising and irradiation laboratory where they are exposed to a limited number of field or calibration sources and their performance compared with those of other participating laboratories. In the present series of intercomparisons one of the objectives has been to involve the participants more fully in the intercomparison by asking them to provide a wide variety of sites for environmental measurements, while the organising laboratories provides administrative and calibration facilities.
A third intercomparison of thermoluminescent dosemeters for environmental measurements has been carried out during 1987 in which the number of measurement sites and the range of calibration facilities have been increased.
In summary, the objectives of this intercomparison were :

a) to provide the participants with the opportunity to have their environmental dosemeters exposed at various and different sites;
b) to enable a quantitative comparison of different types and designs of thermoluminescent dosemeter; and
c) to establish a forum for the discussion of topics of mutual interest in the field of environmental gamma dosimetry.

Report EUR 11870, 1988, 13 pages and 31 tables.

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price : ECU 5

Radiological protection criteria for the recycling of materials from the dismantling of nuclear installations

Recommendations from the Group of experts set up under the terms of Article 31 of the Euratom Treaty.

A considerable fraction of the materials used in the construction of nuclear installations will, on decommissioning and dismantling, be only lightly active or contaminated. These materials could have high economic value and this provides an incentive for recycling or re-use; their recycling either as scrap or as discrete items would require them to follow conventional processing and marking procedures which do not provide for further radiological control.

On the initiative of the Commission of the European Communities, the Group of experts appointed pursuant to Article 31 of the Euratom Treaty set up a Working Party to look into the problem of recycling materials from the dismantling of nuclear installations and to propose appropriate criteria. The Working Party prepared a report which served as a basis for the present recommendations.

A critical review of the situation regarding existing criteria and limits for exemption from regulatory control showed that a suitable basis for establishing criteria for recycling was not available. Two approaches to the problem were explored, one based on defining acceptable individual and collective dose levels, the other based on setting "clearance" levels for the activity concentration of the materials concerned, such that the potential individual and collective doses resulting from recycling would be insignificant. An analysis of current regulatory practice showed that the stipulation of dose limits for recycling poses practical, and sometimes intractable, problems for both regulators and operators. The requisite criteria were consequently formulated based on activity concentration levels. These are directly applicable to steel scrap and equipment from nuclear power plants, but the methods by which they were derived can be applied for the development of criteria for other valuable metals, such as copper and aluminium, and other nuclear installations.

Radiation Protection Series N° 43
Doc. XI/3134/88 EN, 1988, 60 pages

Available on request from :

DG XI/A/1
CEC
Bâtiment Jean Monnet
Rue Alcide Gasperi
L-2920 Luxembourg

Methods used for fixing discharge limits for radioactive effluents from nuclear installations in the Member States

In October 1974, the Commission of the European Communities organised a meeting of representatives of competent authorities and the nuclear power plant operators (UNIPEDE) to review the methods used to determine limits for the discharge of effluents from nuclear power plants in Member States. At this meeting the discussion focussed mainly on discharge authorisation procedures and to a lesser extent, on actual discharge limits.

In April 1977, the Commission organised a further meeting with representatives of the competent authorities and with members of the Group of experts appointed for the purposes of Article 37 of the Euratom Treaty on the generally applicable limits for effluent control and operational discharge limits applied in Member States to nuclear installations. Arising from this meeting the Commission published, in March 1978, a review and analysis of the limits used in the control of radioactive effluents from nuclear installations in the Member States of the European Communities, in the U.S.A., and in some other countries.

Since the above meetings there have been considerable developments as regards both the production of nuclear energy and radiation protection philosophy and standards. The application of the "as low as reasonably achievable" principle in combination with significant technological progress has led to considerable reductions in radioactive effluent discharges from nuclear power plants and nuclear reprocessing complexes. The Commission, therefore, considered that a further meeting involving the competent authorities and the Article 37 Group of Experts to review current practice in establishing discharge limits for nuclear power plants
would be appropriate and such a meeting was held on the 10 and 11 February 1987 in Luxembourg.

The present report is based on both written and verbal submissions made to this meeting together with the various deliberations and discussions which took place. Additional information submitted by some Member States subsequent to the meeting has also been included. Specifically, section 1 surveys and compares the dose limits applied in deriving discharge limits for effluent control in the Member States, whilst section 2 reviews the methodologies and procedures followed in deriving these limits. In section 3 some general conclusions are drawn and finally, in section 4, a summary of a discussion which took place on the relative merits of using dose limits or emission standards is given.

Radiation protection series; n° 42
Doc. XI/3133/88 EN; 1988; 110 pp
Available on request from :

DG XI/A 1
CEC
Bâtiment Jean Monnet
Rue Alcide Gasperi
L-2920 Luxembourg

The cycling of long-lived radionuclides in the biosphere, observations and models.

Proceedings of a workshop co-organized by the Commission of the European Communities and the Centro Investigacion Energetica Medioambiental y Technologica (Madrid)

Madrid (E) 15-19 September 1986.

Long lived radionuclides pose specific problems when their long-term has to be defined, since may eventually enter different biogeochemical cycles. When this cycling is studied, changes occurring over the years in the steady-state of important ecosystems must be also be taken into consideration.

The papers dealing with these cycling problems have been grouped in 3 chapters.

- Biogeochemical cycling of radionuclides in ecosystems.
- Description and modelling of transfer pathways.
- Studies on aquatic and terrestrial compartiments.

Report EUR 1147, 1988, published by CIEMAT press, Madrid, Vol. I + II, 834 pages.

Available on request to :

G. DESMET
DG XII-D-3
CEC
Rue de la LOI 200
B-1049 Brussels

Beta Dosimetry - Fifth Information Seminar on the radiation protection dosemeter intercompraison programme

Proceedings of a Seminar organized by the Commission of the European Communities in collaboration with the ENEA, Bologna (I), 25-27 May 1987

This report contains the account of an intercomparison exercise for personal beta dosemeters which was started in 1986 and completed in early 1987, comments by the participants, a summary of the discussions, various contributions on beta dosimetry, standardisation, quality assurance, dosimetry procedures in Member States, USA and Japan.

Radiation protection series, n° 38

Report EUR 11363 EN, 1988, 307 pages

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price : ECU 25

Radionuclides : A tool for oceanography

Proceedings of an International Symposium jointly organized by the Commission of the European Communities and the "Société Française pour l'Energie Nucléaire" (SFEN) +"l'Institut National des Techniques de la Mer" (INTECHMER-CNAM),

Cherbourg (F), 1-5 June 1987

Edited by J.C. Guary, P. Guegueniat, and R.J. Pentreath.

This book concerns the use of natural and artificial radionuclides as tracers in the oceans as a means by which oceanographic processes can be studied. The research areas of interest vary from worldwide climatology to localised coastal-water pollution studies, from small scale water-mixing to large scale ocean-basin circulation studies, and from processes of exchange across the major interfaces of the oceans to the application of a wide variety of oceanographic models. The book therefore attempts to cover all of the major areas in which radionuclides can be used as tracers in the marine environment.

This volume is divided into 8 chapters.

- Origin and applications or radionuclides in the marine environment.
- Study of large-scale oceanographic processes using naturally occurring radionuclides.
- Naturally occurring radionuclides as tracers of scavenging and particulate transport processes in open and coastal environments.
- Naturally occurring radionuclides in marine sediments and overlying waters.
- Use of artificial radionuclides in the study of water movements in coastal and open ocean systems.
- Artificial radionuclides and sedimentary processes in coastal waters and estuaries.
- Development and application of models to describe the behaviour of radionuclides in the marine environment.

The book is primarily directed to students and researchers in oceanography.

1988; 462 pages; 203 illustrations.

To be ordered through :

Elsevier Applied Science Publishers
Crown House
Linton Road
Barking
GB-Essex IG11 8JU

Price : £ 55.00

Accidental Urban Contamination

Proceeding of a Workshop organized by the Commission of the European Communities in collaboration with the Risø National Laboratory (DK). Roskilde, 9-12 June 1987.

Edited by H.L. GJØRUP, F. HEIKEL VINTHER, M. OLAST and J. SINNAEVE.

Methodologies for evaluating the off-site radiological consequences of hypothetical accidental atmospheric releases of radioactivity are applied in most countries involved in the nuclear fuel cycle. However, over the last decade there has been a considerable evolution in this field and there is now an increasing interest in what is called "Probabilistic Risk Assessment". These probabilistic accident consequence assessment models are used to evaluate the risks presented by accidents in nuclear installations and to provide inputs into decisions on siting, emergency planning and alternative design options. Their use in quantitative decision making framework has implications both for the reliability or precision of the model predictions and for the range of situations for which the models are applicable.

In 1983, the Radiation Protection Research Programme initiated a two-year project on "Methods for Assessing the Radiological Impact of Accidents (MARIA)" and this project is now continued in the frame of the 1985-1989 programme. Key contractors are KfK from the Federal Republic of Germany and NRPB from the United Kingdom. All major European Institutions active in this field also collaborate in a total of 18 contracts. The purpose is to elaborate, by the end of 1989, a generic probabilistic methodology for the evaluation of potential consequences of accidental releases of radioactivity into the environment. Areas for which major uncertainties exist for assessing the consequences of an accident especially concern the urban agglomerations. Indeed, these are characterised by high population densities and consequently contribute substantially to the collective dose commitment.

This Workshop succeeded in accomplishing the following objectives :
- to draw up the state-of-the-art of the implications of an accidental contamination of the urban environment
- to lay down the scientific bases for cost-effective countermeasures and decontamination procedures in the urban environment
- to trigger further experimental work in view of reducing the inherent uncertainties.

Report EUR 11235 EN, published in Radiation Protection Dosimetry, Vol. 21, No 1-3, 1987

To be ordered through:

Radiation Protection Dosimetry
Nuclear Technology Publishing
P.O. Box 7
Ashford
GB-Kent TN25 4NW

Price: £45

Standing Conference on Health and Saftey in the Nuclear age

Proceedings of a conference held in Luxembourg from 5 to 7 October 1987

In their Communication to the Council on the development of Community measures for the application of Chapter III of the Euratom Treaty - Health and saftey (COM(86) 434 final) the Commission of the European Communities announced their intention to initiate a "Standing Conference on Health and Safety in the Nuclear Age" in order to contribute to an increase of information on nuclear activities.

Following this proposition, the Commission (Directorate-General for Employment, Social Affairs and Education, Health and Safety Directorate), organized the first meeting of the Standing Conference in Luxembourg on 5, 6 and 7 October 1987 with the theme "Information for the public and the media on health protection and safety with regard to nuclear activities".

About 120 participants representing scientific experts, the media, the bodies concerned with environmental or consumer protection, the social partners and interested national and international organizations, took part in this conference. It was the first time at European Community level that a meeting allowed an exchange of positions on the health problems related to ionizing radiation by all the parties interested in this subject.

The Commission was asked to pursue this dialogue in order to improve the perception of citizens of the Community of the potential risks and the methods of protection brought into force in the nuclear field.

Radiation Protection Series N° 40

Raport EUR 11608 EN/DE/FR; 1988, 267 pages

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price : ECU 23.75

Reliability of radioactive transfer models

Proceedings of a workshop co-organized by the Commission of the European Communities, the Office of Health and Environmental Research of the US-Department of Energy (USA) and the National Research Centre for Physical Sciences (Greece) and in co-operation with the International Atomic Energy Agency, Vienna. Athens, 5-9 October 1987
Edited by G. DESMET

The assessment of the radiological impact of planned or existing practices involving the actual or potential release of radionuclides into the environment is largely based on the evaluation of modelling techniques which allow prediction of the relationship between environment levels and releases and the associated radiation dose to man. Since models are an imperfect means of representing environmental transfer processes, it is essential to be aware of the reliability which can be associated with the predictions of these models for every assessment situation.

The thirty-seven contributions are grouped in 7 chapters.

- Overview of model reliability and testing studies
- Transfer air-land
- Transfer in terrestrial environment
- Transfer in the aquatic environment fresh water
- Transfer in the aquatic environment marine ecosystem
- Transfer in the biosphere from waste depositories
- Uncertainty analysis

The proceedings are of interest to modellers as well as to field and laboratory researchers. The book is hoped to encourage greater cooperation and exchange of information between these two groups of scientists, usually working in relative isolation from each other.

Report EUR 11367, 1988; 356 pages; 122 illustrations.

To be ordered through :

Elsevier Applied Science Publishers
Crown House
Linton Road
Barking
GB-Essex IG11 8JU,

Price : £ 48.00

Sixth Symposium on Neutron Dosimetry

Proceedings of a symposium jointly organized by the Commission of the European Communities, the GSF (Gesellschaft für Strahlen- und Umweltforschung) Neuherberg (D), co-sponsorded by the US-DOE (Department of Energy) Washington DC and the European Clinical Neutron Dosimetry Group. Neuherberg (D), 12-16 October 1987.

Edited by : H. SCHRAUBE, G. BURGER and J. BOOZ

Emphasis was placed upon all aspects of radiation protection, including results from relevant radiobiological and epidemiological research. Nevertheless, the Sixth Symposium, like the previous ones, acted also as a platform for everybody who is interested and engaged in any physical and technical aspect of neutron production, measurement and application in radiation protection, in biology and medicine.
This proceedings volume contains 108 scientific and technical papers. They are arranged into 16 topics. Most of the topics include invited papers, which are intended to give an introduction and review to the respective field which is then covered in detail by the proffered papers.
Two sections are dedicated to the basic physical data and models which are required for the understanding of the interaction of neutrons with matter. Another one deals with the physical, biological and epidemiological basis of the quality factor for neutrons.
Starting with conceptual considerations, the first six sections cover aspects of radiation health physics problems. Here, the majority of the contributions consider problems of practical neutron measurements using solid state nuclear track detectors and tissue equivalent proportional counters.
The subsequent six sections are concerned with intrumentation for neutron beam dosimetry, as well as with the installations to produce neutron beams for radiobiological experiments and for medical application in high LET radiotherapy.

Report EUR 11663, published in Radiation Protection Dosimetry, Vol.23, Nos 1-4, 1988, 498 pages.

To be ordered through :

Nuclear Technology Publishing
P.O. Box Nr 7
Ashford
GB-Kent

Price : £ 140

Results of the third CEC intercomparison of active and passive detectors for the measurement of radon and radon decay products

Proceedings of a Workshop co-organized by the Commission of the European Communities and the National Radiological Protection Board (GB), Chilton (GB), 19-23 October 1987

Edited by J. MILES and J. SINNAEVE

The third intercomparison on the European region was carried out in two stages, the first for passive integrating detectors and the second for active instruments, usually by spot measurements. Twenty-one laboratories participated in the intercomparison of passive dosimetry and seventeen in that for active dosimetry. Passive detectors have been used in many European countries to carry out surveys of exposure in homes and to monitor occupational exposure. The exposures for the intercomparison of passive detectors were carried out in June, July and August 1987 at NRPB. The intercomparison of active detectors was carried out over three days in October 1987, when scientists from the participating laboratories brought their equipment to NRPB.

The results of this intercomparison and the two previous ones haVE shown that standards of radon and decay product metrology steadily improved. Detailed results are given.

Report EUR 11882 EN, 1988, 77 pages

To be ordered through :

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price (excluding VAT) in Luxembourg : ECU 7.50

Fourth International Symposium on the Natural Radiation Environment

Proceedings of a Workshop co-organized by the Commission of the European Communities, the National Laboratory of Engineering and Industrial Technology of Portugal and the Office of Health and Environmental Research of the United States Department of Energy. Lisbon (P), 7-11 December 1987.

Edited by A.O. DE BETTENCOURT, J.P. GALVAO, W. LOWDER, M. OLAST and J. SINNAEVE

The major achievements are outlined as follows :

Atmospheric and aerosols studies

The methodology for size measurements of the decay has been considerably improved; it is recognised that the "unattached" fraction is really a highly diffuse mode in the size distribution. Important progress has been made in the understanding of change transfer and ion clustering effects on the behaviour of the radon decay products.

Radon Behaviour

Substantial progress has been made on understanding the entry of radon into the indoor environment and on the relationships between indoor concentrations and influencing factors, including soil or geological, structural, meteorological and operational factors.

Dose and risk assessment studies

It is believed that the proportional hazard model enables a more reliable risk projection from lung cancer than absolute projection models. A dose conversion factor of 1mSv per 20 $Bq.m^{-3}$ was proposed.

Control strategies and policies

There is a growing awareness and emphasis through the world that radon is a public health issue. There has been much scientific and technical information developed to help support the conception, development and implementation of national strategies and policies for control of exposure to natural radioactivity.

Report EUR 11895, EN, published in Radiation Protection Dosimetry, Vol. 24, Nos 1-4, 1988, 560 pages

To be ordered through :

Nuclear Technology Publishing
P.O. Box N° 7
Ashford
GB- Kent TN25 4NW

Price : £ 50

Recent advances in reactor accident consequence assessment

Proceedings of a workshop co-organized by the Commission of the European Communities, the Nuclear Energy Agency of the OECD and the Direzione Sicurezza Nucleare e Protezione Sanitaria of the ENEA (I).
Rome (I), 25-29 January 1988

Edited by M. OLAST and J. SINNAEVE

A review of the present knowledge of probabilistic accident consequence assessment techniques and their applications. This includes the atmospheric dispersion and deposition modelling, with comparison of the different approaches, the exposure pathways with emphasis on post deposition processes, the health effects with emphasis on the consequences of the Hiroshima and Nagasaki data re-evaluation, the countermeasures and their economic consequences, the uncertainty analysis of the models and finally the applications of these models as a help to decision making.

Report EUR 11408, 1988; 464 pages; 147 figures; 80 tables.

To be ordered through:

Office for Official Publications
of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 37.50

Technical and Physical Parameters for Quality Assurance in Medical Diagnostic Radiology : Tolerances, Limiting Values and Appropriate Measuring Methods

Proceedings of a Workshop organized by the Commission of the European Communities, Brussels (B), 23-25 February 1988.

Edited by B.M. MOORES, F.E. STIEVE, H. ERISKAT, H. SCHIBILLA

Within the framework of its Radiation Protection Programme, the Commission of the European Communities participates in research which is aimed at the reduction of patient exposure during medical diagnostic radiology. This Community action is directly related to the Council Directive of 3 September 1984 laying down basic measures for the radiation protection of persons undergoing medical examination or treatment. Article 3 of this Directive stipulates that :
"... All installations in use must be kept under strict surveillance with regard to radiological protection and the quality control of appliances."
As a consequence of this, numerous quality assurance measures have been initiated by many radiological departments to cover the quality control of all those parameters which are important for equipment functioning, imaging methods and image interpretation.

In order to compare and evaluate the results of these individual efforts, radiologists, radiographers, research workers in radiation protection and medical physics, representatives from related industries, public health authorities and regulating bodies discussed the requirements governing measuring methods, instrumentation and measuring techniques used for the quality control of those parameters which have the greatest impact on the quality of the radiographic images and patient exposure.

The 51 papers in the Proceedings deal with a variety of subject areas such as : organizational aspects of quality control, parameters of the image producing system using conventional x-ray equipment or special equipment and techniques, image recording systems - system analysis as well as image performance, retrieval - viewing conditions, and dosimetry. Increasing attention was given to special techniques like mammography, computed tomography and digitized radiography.
Efforts were made to find commonly acceptable tolerances for different measuring methods and instruments and to define or to fix limiting values for the most significant parameters. The possibilities were discussed to standardizing those tolerances and limiting values in order to maintain consistently high performance of radiological equipment and image processing. The need for intercomparison of the various quality test methods was stressed so that the prerequisites for the production of comparable radiographs without unnecessary exposure of the patient will result.

Report EUR 11620, Br. Inst. Radiol. Report Nr 18, about 160 pages, in press

To be published by :

British Institute of Radiology
36 Portland Place
GB-London W1N 4AT
Price : £ 30

Biological assessment of occupational exposure to actinides

Proceedings of a workshop jointly organized by the Commission of the European Communities and the Institut de Protection Nucléaire, C.E.A. Fontenay-aux-Roses (F), Versailles (F), 30 May - 2 June 1988

Edited by: G.B. GERBER, H. METIVIER, J.W. STATHER, R.G. THOMAS

The problems related to setting annual levels of intake and to monitor exposure from actinide material used in the nuclear industry formed the subject of the workshop. The methods of determining exposure from excretion values, the modelisation of actinide behaviour in the organism, the biological consequences of exposure to uranium, plutonium and other actinides as well as the possible means to deal with over-exposure were reviewed. A round table discussion, which is also summarised in the proceedings, reviewed the problems involved in the radiation protection of persons exposed to such compounds.

Report EUR 12087 EN, in press

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Implementation of dose-equivalent operational quantities into radiation protection practice

A Seminar jointly organized by the Commission of the Europan Communities and the Physikailsch-Technische Bundesanstalt (PTB) and co-sponsored by ICRU (The International Commission on Radiation Units and Measurments), ICRP (The International Commission on Radiological Protection), the US-Department of Energy and EURADOS (The European Radiation Dosimetry Group). Braunschweig (D), 7-9 June 1988

Edited by J. BOOZ and C. DIETZE

The selection of quantities to be used in radiation protection has been a problem since the time, people become aware of radiation protection to be necessary. In 1977, the ICRP proposed the quantities "effective dose equivalent" a,d "mean dose equivalent to a particular organ" to be the basis for primary limits in radiation protection against ionizing radiation. These quantities are not directly measurable and as a consequence, operational quantities must therfore be introduced which are related to the limiting quantities in the sence that they serve as an upper assessment in most cases and can be used in practical applications. In 1985, the ICRU proposed a system of dose equivalent operational quantities for external exposure to be used in area and individual monitoring of any ionizing radiation.
Meanwhile, in many countries investigations have been performed to study the applicability of the new quantities and the consequences with respect to instrumental requirements and calibration procedures.

The Proceedings concentrate on the following main topics :
- Problems of introducing the dose equivalent operational quantities into practice
- Determination and discussion of conversion factors between quantities
- Practical assessment and calculation of operational and calibration quantities
- Calibration procedures for instruments used, in particular for individual monitoring
- Instrumental requirements with regard to energy and angular responses
- Practical experience in the performance of individual dosimetry systems concerning the operational quantities

The discussion during the Seminar showed that with respect to area monitoring a broad international acceptance of the operational quantities "ambient dose equivalent" and "directional dose equivalent" has been achieved. The implementation into radiation protection practice, however, is still at the beginning.
For individual dosimetry, many new experimental and calculated data have been presented during the Seminar which gave strong impact to the discussion. A general international acceptance of the proposed operational dose equivalent quantities in this field, however, has not been achieved, to date, and further discussion is necessary.

In press

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Critical evaluation of Radiobiological data for biophysical monitoring

Report of a Workshop jointly organized by the Commission of the European Communities and the US-Department of Energy. Oak Ridge, Tennessee, (USA) 23-25 June 1988.

The Department of Energy's Office of Health and Environmental Research and the Commission of the European Communities Radiation Protection Programs support the majority of Research in the Field of Radiobiological Modeling. This field of science develops models based on scientifically sound bases to predict biological response (at the cellular, molecular, and animal level) to exposure to low level ionizing radiation. A Workshop was held to review the data available from physical and chemical, cellular and molecular and animal studies with ionizing radiation. A number of leading experts participated at this workshop and after identification of the available data they attempted to evaluate two categories. First, data that are generally considered quite good and most relevant for radiobiological modeling and, second, data that are needed in the future for modeling. To provide a common basis for comparing various radiobiological models, it was considered important to identify data that were generally acceptable by most modelers. Organizers believe that this objective of the workshop was mostly achieved. Three working groups representing the fields of physics and chemistry, cellular and molecular and animal studies were formed, and prepared a summary of the workshop and a list of references for the data in each field. The report presents this summary.

1988, 54 pages, in press

Available on request from :

Office of Health and Environmental Research
US-Department of Energy
Washington, DC 20545
USA

Low dose radiation effectiveness and mechanisms of high-LET particles

Proceedings of a Workshop jointly organized by the Commission of the European Communities and the US-DOE (Department of Energy) - Washington DC. Darmstadt (D), 16-17 July 1987.

The contribution of research on high-LET particles to the problem of neutron effectiveness; listing of new and innovative concepts from high-LET research that can lead to improved understanding of fundamental mechanisms; specification of topics for long-term research on high-LET radiation problems and their priorities

Document Nr XII/273/88, 1988, 7 pages

Available on request to :

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Rue de la Loi 200
B-1049 Brussels

Applications, perspectives and limitations of comparative risk assessment and risk management

Proceedings of a Workshop co-organized by the Commission of the European Communities and the Centre de Développement des Etudes et Applications en Hygiène et Sécurité (CEDHYS), France. Nice (F), 26-30 September 1988

Edited by G. BRESSON, M. OLAST and J. SINNAEVE

The main topics discussed were :
- Aims of comparative risk assessment studies
- Input data and their uncertainties
- Methods and their assumptions
- Case studies at different levels
- Limits of validity
- Risk management strategies
- Risk perception
- Future perspectives

The experiences and knowledge as well as methodologies developed and acquired in radiation protection research for assessing risks to man and his environment are now known to be applied in a broader context to prevent, reduce and manage risks linked to numerous human industrial activities. The apparent need to compare such risks and the increasing concern of the authorities responsible for the environment and for the safety and well-being of the population and the workers urged the scientific community to develop methods for risk management based upon a comparative approach.

Report EUR 11465,EN, in press

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Optimization of Image Quality and Patient Exposure in Diagnostic Radiology

Proceedings of a Workshop organized jointly by the Commission of the European Communities and the National Radiological Protection Board - Chilton, Oxford, 27 - 29 September 1988

Edited by B.M. MOORES, B. WALL, H. ERISKAT, H. SCHIBILLA

The purpose of the Workshop was to formulate criteria for optimizing diagnostic radiology from the standpoint of image quality and patient exposure and to weigh these factors against each other. Some of the 77 papers deal with problems of image perception and interpretation and the quantification of image quality. A remarkably wide range of test objects for the evaluation of image quality is described.

From the standpoint of optimization, the requirements for a series of clinical situations are considered in detail. Considerable attention is paid to mammography and paediatric radiology.

Other areas such as chest examinations, urography and dental radiology provide evidence of similar avoidable variations in patient exposure and image quality.

Surveys of trends in diagnostic radiology over considerable time periods in Canada, Sweden and southern Germany have proved that regular quality checking contributes in a large measure to restricting dose ranges and reducing both the mean patient dose and the number of unacceptable radiographs.

The urgency with which quality criteria must be established is also stressed with reference to examinations conducted by means of computed tomography and digital radiography.

A CEC Study Group composed of specialists in radiology, medicine, medical physics and equipment technology has drawn up a list of quality criteria for six common diagnostic examinations : chest, skull, lumbar spine, pelvis and sacrum, urinary tract and breast. These criteria were checked on approximately 1000 patients in 10 European countries and the results are presented and compared with those of other approaches aimed at better image quality and lower patient exposure.

This should constitute a scientific contribution to a conceivable standardization of quality criteria and to the production of comparable radiographs, perhaps even of the "Euro X-ray". The revised list of quality criteria is appended to the Proceedings.

Report EUR 11842, Br. Inst. Radiol. Report Nr 20, about 180 pages, in press

To be published by :

British Institute of Radiology
36 Portland Place
GB-London W1N 4AT

Risks from Radium and Thorotrast

Proceedings of a workshop jointly organized by the Commission of the European Communities, the U.S. National Cancer Institute and the U.S. Department of Energy, Office of Health and Environmental Research
Bethesda (USA), 3-5 October 1988

Edited by: D.M. TAYLOR, C.W. MAYS, G.B. GERBER, R.G. THOMAS

The workshop considered the epidemiological and experimental data dealing with the effects of radium and thorotrast. Risk assessment for cancer and non-stochastic damage for internally deposited radionuclides rests primarily on information obtained from these two alpha emitters. The papers presented updates of the epidemiological studies in different countries (ie Denmark, Germany, Japan, UK and USA), new information on dosimetry of cells at risk and on ongoing animal studies, and attempts to extrapolate the data to other exposure situations. Two round table discussions, published also in summary, dealt with the respective risks of radium and thorotrast.

Report EUR 12088 EN, supplement to the British Journal of Radiology No. 20, in press

To be published by:

The British Institute of Radiology
36 Portland Place
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Price: £ 40.00

Implementation of dose equivalent meters based on microdosimetric techniques in radiation protection

Proceedings of a Workshop jointly organized by the Commission of the European Communities and the GSF (Gesellschaft für Strahlen- und Umweltforschung), Neuherberg, in co-operation with EURADOS (European Dosimetry Group).
Schloss Elmau (D), 18-20 October 1988

Edited by H. MENZEL, J. BOOZ and H.G. PARETZKE

Microdosimetric low pressure tissue-equivalent proportional counters (TEPC) have played for many years an important role in theoretical and experimental radiation research. Development and initial experience gained with dose equivalent (rate) meters based on this TEPC technique in radiation protection were discussed already in 1984 at a first CEC-Workshop at Homburg. Since that time microdosimetric instruments for area and individual dosimetry have been used to measure dose equivalent (rate) in many fields of practical interest. Further on, two experimental intercomparisons have been performed in several neutron reference fields at the Physikalisch-Technische Bundesanstalt, Braunschweig, in 1986 and 1987.

In this second Workshop at Schloss Elmau the results of these intercomparisons were discussed, and information on experience obtained under practical radiation protection conditions with respect to response, sensitivity and performance of such TEPC dose equivalent meters was exchanged. In addition, the basic and practical aspects of requirements for the performance of such instruments for area and individual monitoring were reviewed. Twenty-eight papers were given in nine sessions on :

- Development of new instruments for experimental microdosimetry
- Basic physical aspects of proportional counter measurements
- Intercomparisons of TEPC instruments
- TEPC measurements in photon radiation fields
- Practical use of TEPC dose equivalent meters at working places
- Numerical methods and improvement of TEPC response to neutrons
- Space radiation dosimetry
- Specification of radiation quality
- Requirements for the performance of dose equivalent meters in area and individual monitoring

Due to those papers and extensive time for discussion the participants could fully achieve the purpose of this Workshop, namely to review the state-of-the-art of this technique, to outline important future experimental and theoretical work, and to indicate the limitations and advantages of TEPC based dose equivalent meters in radiation protection.

In press

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OTHER PUBLICATIONS

Catalogue of contracts of the Commission's Radiation Protection Programme 1985-1989

This catalogue has been published in two volumes : Volume I containing information on the management data such as contractor, subject of the research projects, duration, budget, etc... and Volume II containing the scientific description of each project. The aim pursued through this publication is to convey a better transparency of the Commission's programme and to serve as an aid for its management.

This first edition (July 1987) represents some 228 contracts covering more than 340 research projects. It reflects the situation on 31.12.1986.

An updating of this catalogue of contracts of the Radiation Protection Programme 1985-1989 will be published in the course of 1989.

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VI

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LIST OF RESEARCH GROUP LEADERS

INDEX DES CHEFS DE GROUPE DE RECHERCHE

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** Bericht noch nicht verfügbar
Report not yet available
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Europäische Gemeinschaften — Kommission
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EUR 12064 – Tätigkeitsbericht — Programm Strahlenschutz — 1988
Progress report — Radiation protection programme — 1988
Rapport d'activité — Programme radioprotection — 1988

Luxembourg: Office for Official Publications of the European Communities

1989 – X, 1 963 pp. – 16.2 × 22.9 cm

Radiation protection series

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ISBN 92-825-9995-7

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The progress report of the radiation protection programme outlines the research work carried out in 1988 under contracts between the Commission of the European Communities and research groups in the Member States. Results of more than 350 projects are reported. They are grouped into six sectors: Radiation dosimetry and its interpretation; Behaviour and control of radionuclides in the environment; Non-stochastic effects of ionizing radiation; Radiation carcinogenesis; Genetic effects of ionizing radiation; Evaluation of radiation risks and optimization of protection.

Within the framework programme, the aim of this scientific research is to improve the conditions of life with respect to work and protection of man and his environment and to assure a safe production of energy, i.e.:

(i) to improve methods necessary to protect workers and the population by updating the scientific basis for appropriate standards;
(ii) to prevent and counteract harmful effects of radiation;
(iii) to assess radiation risks and provide methods to cope with the consequences of radiation accidents.

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