COMMISSION OF THE EUROPEAN COMMUNITIES

EURATOM

RADIATION PROTECTION

PROGRAMME

PROGRESS REPORT

1987

EUR 11464 DE/EN/FR

KOMMISSIONEN FOR DE EUROPÆISKE FÆLLESSKABER
KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN
COMMISSION OF THE EUROPEAN COMMUNITIES
COMMISSION DES COMMUNAUTÉS EUROPÉENNES
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COMMISSIE VAN DE EUROPESE GEMEENSCHAPPEN

EURATOM

Beretning
Program

STRÅLINGSBESKYTTELSE

Tätigkeitsbericht
Programm

STRAHLENSCHUTZ

Progress report

RADIATION PROTECTION

programme

Rapport d'activité
Programme

RADIOPROTECTION

Rapporto d'attività
Programma

RADIOPROTEZIONE

Verslag van de werkzaamheden
Programma

STRALINGSBESCHERMING

1987

CI-**EUR 11464 DE/EN/FR**

Published by the
COMMISSION OF THE EUROPEAN COMMUNITIES

Directorate-General
Telecommunications, Information Industries and Innovation

Bâtiment Jean Monnet
LUXEMBOURG

Bibliographische Daten befinden sich am Ende der Veröffentlichung.
Cataloguing data can be found at the end of this publication.
Une fiche bibliographique figure à la fin de l'ouvrage.

Luxembourg : Office for Official Publications of the European Communities, 1988

ISBN 92-825-8337-6 Kat./cat. : CD-NA-11464-3A-C

Printed in Belgium

I N H A L T S V E R Z E I C H N I S

T A B L E O F C O N T E N T S

T A B L E D E S M A T I E R E S

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* Schlussbericht
Final report
Rapport final

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

I

EINLEITUNG

INTRODUCTION

INTRODUCTION

VORWORT

Der vorliegende Tätigkeitsbericht 1987 des Strahlenschutzprogramms der Kommission der Europäischen Gemeinschaften gibt einen Überblick über die Ergebnisse von rund 320 einzelnen Forschungsprojekten und verweist auf etwa 600 wissenschaftliche Veröffentlichungen. Eine Reihe von Forschungsarbeiten haben sich mit den radiologischen Folgen des nuklearen Unfalls von Tschernobyl befasst und weitere Forchungsvorhaben wurden vorbereitet, für die die erforderlichen Mittel durch den Beschluss des Ministerrats vom 21. Dezember 1987 bereitgestellt wurden, mit dem die Revision des Strahlenschutzprogramms angenommen wurde. Die vorrangigen Themen beziehen sich auf den Transfer von Radioaktivität durch die verschiedenen Bereiche der Umwelt, Behandlung von Strahlengeschädigten, Massnahmen um die radioaktive Kontaminierung herabzusezten und wissenschaftliche Grundlagen für die Aufstellung abgeleiteter Notfall-Bezugswerte.
Gesteigerter Wert wird auf Koordinierung der Forschungsarbeiten und auf umfangreichen Informationsaustausch gelegt, wodurch auch das Strahlenschutzprogramm dazu beizuträgt, ein Europa der Forscher zu schaffen. So trafen sich 1987 Vertagspartner und eingeladene Sachverständige in 26 Studiengruppen, um ihre Forschungspläne aufeinander abstimmen und eine Zusammenarbeit zu stimulieren. 30 Workshops und Seminare wurden organisiert, um Probleme und Lösungsansätze zu diskutieren für Schwerpunktsthemen wie : Sofort- und Spätwirkungen nach Strahlenbelastung, Strahlenempfindlichheit des sich entwickelnden Organismus, Dosimetrie : Messungen und ihre Interpretation, Wirkungen niederer Strahlendosen, Übertragungsmodelle der Radioaktivität in der Umwelt, natürliche Strahlenbelastung, Abschätzung der Folgen nuklearer Unfälle, Interventionswerte für die radioaktive Kontaminierung von Nahrungsmitteln und Information der Öffentlicheit (s. Kapitel IV).

Die Veröffentlichung von Sitzungsberichten und Monographien erweitert den Kreis der angesprochenen Wissenschaftler erheblich (s. Kapitel V).

Der Wunsch auch Länder ausserhalb der Europäischen Gemeinschaft in diese Bestrebungen einzubeziehen, führte zu der Unterzeichnung einer Absichtserklärung mit der Atomic Energy of Canada Ltd, mit der bestimmte gemeinsame Forschungsziele im Strahlenschutz definiert wurden und eine Koordinierung der entsprechenden Arbeiten angestrebt wird. Ausserdem wurde die bereits laufende Zusammenarbeit mit dem Department of Energy der Vereinigten Staaten von Amerika intensiviert.

S. FINZI
Direktor
Forschung über nukleare Sicherheit

W. HUNTER
Direktor i.V.
Gesundheit und Sicherheit

FOREWORD

The 1987 Progress Report of the Radiation Protection Programme of the Commission of the European Communities provides an overview of the results of about 320 individual research projects resulting in approximately 600 scientific publications. A series of research studies concentrated on the radiological consequences of the nuclear accident at Chernobyl and further research projects have been prepared using the financial means which were made available by the decision of the Council of Ministers on 21 December 1987, adopting the Revision of the Radiation Protection Programme. The priority topics refer to the transfer of radioactivity through the various components of the environment, treatment of irradiation victims, measures to reduce the radioactive contamination, scientific basic data for the derivation of emergency reference levels.

Increased importance is being given to coordination of research work and comprehensive information exchange, contributing also in the frame of the Radiation Protection Programme to the creation of a Europe for research workers. Thus, in 1987, meetings of 26 study groups were held, at which the contracting parties and invited experts sought to harmonize their research plans and to stimulate cooperation. At 30 Workshops and Seminars, problems and solutions were discussed in key subjects such as acute and late effects after irradiation, radiosensivity of the developing organism, dosimetry: measurements and their interpretation, effects of low doses, models of radioactivity transfer in the environment, natural radiation exposure, assessment of consequences of nuclear accidents, intervention levels for the radioactive contamination of foodstuffs and information for the public (see Chapter IV).

The publication of proceedings and monographs increases considerably the number of scientists brought into contact with the Programme actions (see Chapter V).

The desire to include countries outside the European Community in these endeavours resulted in the signing of a Memorandum of Understanding with the Atomic Energy of Canada Ltd, defining some common research objectives in the field of radiation protection and establishing ways of coordinating work in this area. Furthermore, the previously established cooperation with the US-Department of Energy has been intensified.

S. FINZI
Director
Nuclear Safety Research

W. HUNTER
Acting Director
Health and Safety

PREFACE

Le présent rapport d'activités 1987 du Programme Radioprotection de la Commission des Communautés européennes donne un aperçu des résultats de plus de 320 projets de recherches repris dans quelque 600 publications. Une série de travaux a été consacrée à l'étude des conséquences radiologiques de l'accident nucléaire de Tchernobyl, et d'autres projets ont été préparés pour lesquels des moyens financiers nécessaires ont été accordés par la décision du Conseil des Ministres le 21 décembre 1987, adoptant la révision du Programme Radioprotection. Des sujets prioritaires concernent le transfert de la radioactivité par les divers composants de l'environnement, le traitement des victimes d'accidents nucléaires, les mesures pour réduire la contamination radioactive et les bases scientifiques pour l'établissement des niveaux dérivés de références en cas d'urgence.
Une importance accrue est accordée à la coordination des travaux de recherche et aux échanges d'informations. Le Programme Radioprotection contribue ainsi, lui aussi, à la création d'une Europe des chercheurs. C'est dans cet esprit qu'en 1987 les partenaires contractuels et des experts invités se sont rencontrés dans 26 groupes d'études afin de concerter leurs plans de recherche et de stimuler la coopération. Trente ateliers et séminaires ont été organisés, afin de discuter des problèmes et des recherches nécessaires concernant des thèmes clés tels que les effets à court et à long terme après irradiation, la sensibilité aux radiations de l'organisme en voie de développement, la dosimétrie : les mesures et leur interprétation, les effets des irradiations à faibles doses, les modèles de transfert de la radioactivité dans l'environnement, l'irradiation naturelle, l'évaluation des conséquences d'accidents nucléaires, les interventions en cas de contamination radioactive des denrées alimentaires et l'information du public (voir chapitre IV).
La publication de comptes rendus et de monographies contribue considérablement à agrandir le cercle des chercheurs touchés par ces actions (voir chapitre V).
Le désir d'élargir la coopération avec des pays en dehors de la Communauté européenne a conduit à la signature d'une déclaration d'intentions avec l'Atomic Energy of Canada Ltd définissant des objectifs communs dans certains secteurs de recherche en radioprotection et visant une coordination des travaux nécessaires pour atteindre ces objectifs. De plus, la coopération déjà entamée avec le Department of Energy des Etats-Unis d'Amérique a été intensifiée.

S. FINZI
Directeur
Recherche Sécurité Nucléaire

W. HUNTER
Directeur a.i.
Santé et Sécurité

II

Mitglieder und Experten 1987

Beratender Verwaltungs- und Koordinierungsausschuss "STRAHLENSCHUTZ"

Members and experts 1987

Management and Coordination Advisory Committee "RADIATION PROTECTION"

Membres et experts 1987

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

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

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A. LAFONTAINE
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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

1987

Contractor: Contract no.: BI6-A-001-F

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

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 Cédex

Dr. M. Terrissol
Centre de Physique Atomique
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:

Les programmes de calcul que nous mettons au point pour le transport des neutrons permettent de calculer la fluence particulaire Φ_i en neutrons dans le détecteur pour chaque tranche d'énergie et par neutron source (voir dernier rapport 1986). Ceci nous permet d'évaluer le Kerma dans le modérateur :

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

où n est le nombre de tranches d'énergie prises en considération et k_i le facteur de conversion fluence-kerma.

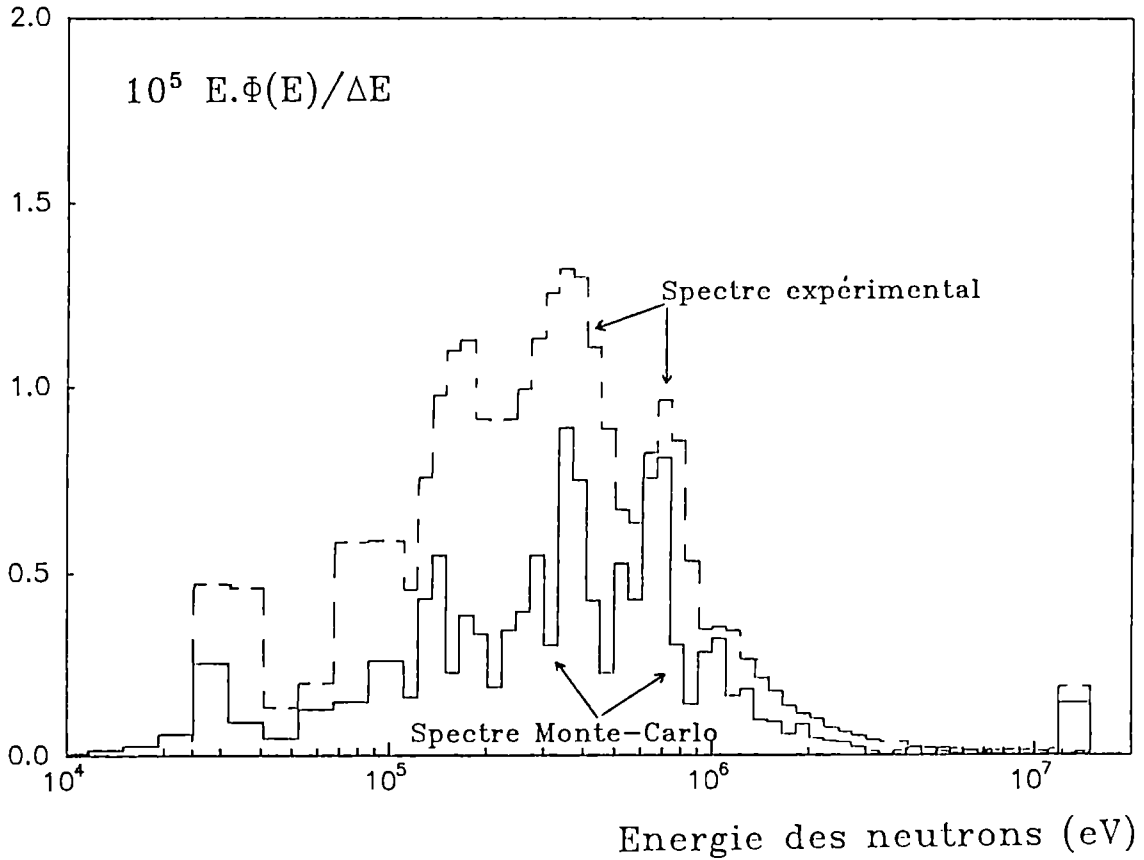
En comparant les résultats obtenus par le code de calcul et les mesures effectuées à l'aide de chambres d'ionisations et de compteur Geiger à Cadarache, nous avons constaté une sous-estimation d'un facteur 2 par le calcul. Ceci nous a amené à prendre en considération d'une manière plus précise les formes du convertisseur d'uranium vers les rétrodiffusés et ainsi tenir compte de l'émission 4π et des boucliers de fer. De même, le calcul et l'expérience ont montré que l'on pouvait négliger l'air entre le bouclier de fer et le modérateur, ainsi que le chariot de fer ; ce qui a permis un gain de temps de calcul non négligeable.

Nous pouvons voir sur la figure, la comparaison des résultats expérimentaux et du code de calcul après ces modifications. 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. Avec ces corrections le Kerma (calculé à 50 cm) est :

17.10^{-17} Gray/neutron par le code Monte-Carlo

26.10^{-17} Gray/neutron par mesure expérimentale

le facteur est passé de 2 à 1,5 et nous nous efforçons de rechercher et de remédier à cette différence.


2.0
1.5
1.0
0.5
0.0
10^5 E.Φ(E)/ΔE
Spectre expérimental
Spectre Monte-Carlo
10^4
10^5
10^6
10^7
Energie des neutrons (eV)

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

1967

Contractor:

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

Contract no.: BI6-A-180-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 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. Simulation of low-energy electron transport as a function of time. Application to microdosimetry and radiobiology.

Title of the 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(s) 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:

Achievement of the Monte-Carlo type code which solves the equation of diffusion for the chemical evolution of species created in liquid water.

III. Progress achieved.

We have developped a Monte-Carlo code to simulate the chemical stage following water radiolysis by low energy electrons. The model tries to solve the differential system :

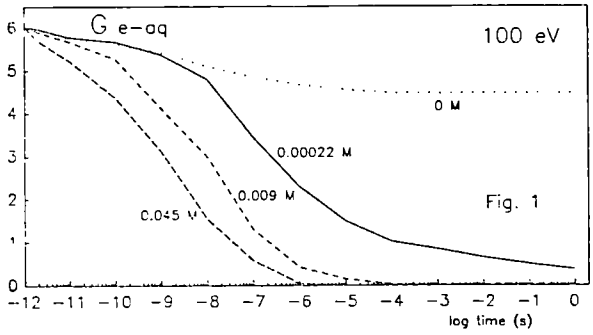
$$\partial C_i / \partial t = D_i \nabla^2 C_i - \Sigma_j k_{ij} C_i C_j$$

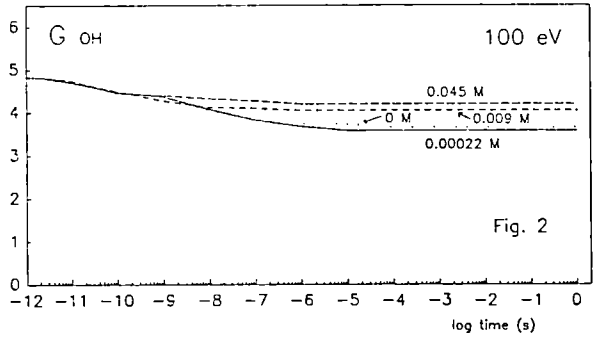
here the C_i, the concentrations of species i, are a function of space and time, D_i are the diffusion constants and k_{ij} the rate of reaction between species i and j. In the case of pure irradiated water, we have species e^-_{aq}, H, OH, H_2O_2, H^+_{aq}, H_2, OH^-, H_2O and about 20 possible reactions between them.

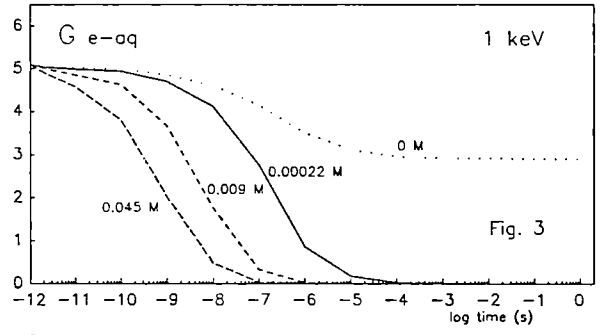
Space and time evolution starting at 10^{-12} second are done according to SMOLUCHOWSKY law and DEBYE equation. Among lots of routines, to spare computer time, for instance, (in order to reach times of about 1 second after irradiation) we have built a program which splits, as many times as necessary the space distribution of species into "clusters". A cluster is a group of species able to react during a time step, but unable to react with any species of any other cluster.

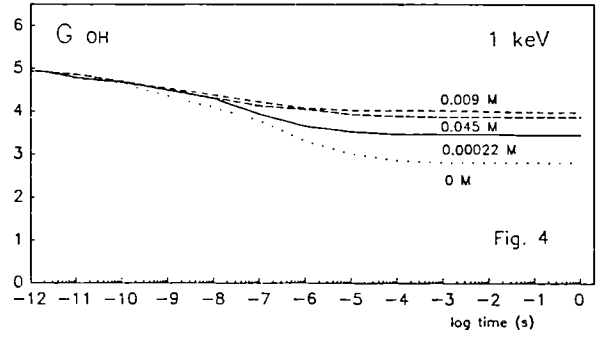
We are able to apply our set of programs to the simulation of species evolution created by electrons with initial energies ranging from 50 eV to 10 keV. We have begun to introduce other species, impurities or scavengers and the corresponding set of reactions in our programs and the first obtained results are promising. For instance we can see on figures the effect of dissolved oxygen for 100 eV and 1 keV incident electron in liquid water. Fig. 1-2 represents G values for e^-_{aq} and OH radical for various O_2 concentrations and 100 eV incident electrons. Fig. 3-4 are for 1 keV incident electron.

For low energy photons, we are able now to simulate their transport down to about 10 eV with a new set of photoionisation cross-sections and numerical technics to obtain the differential ones. The achievement of the photon-electron cascade will be done next year.


G e−aq
100 eV
0 M
0.00022 M
0.009 M
0.045 M
Fig. 1
log time (s)


G OH
100 eV
0.045 M
0 M
0.009 M
0.00022 M
Fig. 2
log time (s)


G e−aq
1 keV
0 M
0.00022 M
0.009 M
0.045 M
Fig. 3
log time (s)


G OH
1 keV
0.009 M
0.045 M
0.00022 M
0 M
Fig. 4
log time (s)

IV Objectives for the next 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 code down to about 10 eV in gazes and liquid water.

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

VI. Publications:

M. TERRISSOL, A. BEAUDRE, V. CAUDRELIER : "Simlulation of spatial temporal evolution of chemical species created by electrons and photons in liquid water". Proceedings of the 8th International Congress of Radiation Research, EDIMBURG July 1987, Taylor & Francis editors, p. 48.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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

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.: 1:

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 and 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:

From previous studies with thimble type tissue-equivalent (TE) and Al high-pressure ionization chambers operated at pressures up to about 8 MPa with several filling gases, it was concluded that initial recombination increases with increasing radiation quality and that the relative reading (reading with reference to atmospheric pressure) can be increased. The studies for 2.1 MeV neutrons are aimed at developing a sensitive system for determination of total dose (equivalent) and photon dose for a large energy range and at obtaining information on cavity size effects and initial recombination as a function of neutron energy.

III. Progress achieved.

For 2.1 MeV neutrons, produced by the p(2.9)+T reaction, the relative readings (defined as the ionization chamber reading at pressure p and a collecting potential of 600 V relative to that at 0.1 MPa and 600 V) as a function of the pressure of various gases are shown in Figures 1 and 2 for the TE and Al chambers, respectively. For CH_4 in the TE chamber and CH_4, TE-CH_4 and C_2H_4 in the Al chamber at lower pressures the increase in the relative reading is more rapidly than proportional with the pressure. For CH_4 and TE-CH_4 gas an increase in the relative reading is observed up to a maximum followed by a decrease. A similar observation is most likely valid for C_2H_4 when the measurements are extended to higher pressures. The maxima observed in the relative reading with the different chambers differ in value and are reached at different pressures for CH_4 and TE-CH_4. For Ar a continuous increase of the relative reading with pressure is found.

An increase of the gas pressure results in an increase of the mass of the gas in the chamber. As a first consequence, the ionization due to charged particles created in the wall increases with pressure, but secondly, also the amount of secondary charged particles produced in the gas increases with pressure. Consequently, an increase more rapidly than proportional to the pressure is to be expected when the contribution to the ionization from charged particles produced in the gas is significant and ion recombination is not too large. Among the investigated gases containing hydrogen, the probability for interaction with 2.1 MeV neutrons is largest and ion recombination is lowest for CH_4. Consequently, the largest increase of the relative reading with pressure is observed for CH_4. The more rapid increase observed for the Al chamber is expected since Al has a smaller cross section for 2.1 MeV neutrons.

A comparison of the relative readings of the TE and Al high-pressure chamber with CH_4 for 0.9 and 2.1 MeV neutrons shows that the maximum relative readings increase with increasing neutron energy (Al chamber: 30 and 110; TE chamber: 16 and 25 for 0.9 and 2.1 MeV neutrons, respectively). The pressures at which the maxima are reached, increase with neutron energy (2 MPa and 4 MPa for 0.9 and 2.1 MeV neutrons, respectively) and the ratio of the maximum relative readings with the Al and TE chamber increases with neutron energy (about 1.9 and 4.4 for 0.9 and 2.1 MeV neutrons, respectively). The recombination parameter R(500V)/100V) at about 1 MPa of 1.42 for 2.1 MeV neutrons is smaller than the corresponding value of 1.49 for 0.9 MeV neutrons.

Assuming that the reading at 0.1 MPa in the Al chamber is completely due to secondary charged particles created in the gas, it is estimated for the TE chamber with CH_4 gas that at 1 bar the contributions to the total ionization of secondary charged particles created in the wall are about 0.47 and 0.77 for 0.9 and 2.1 MeV neutrons, respectively.

The dependence of the relative reading with Ar in the TE and Al chambers for 2.1 MeV neutrons is qualitatively similar to that for 0.9 MeV neutrons, mainly due to photons and can be used to derive the photon component of the total absorbed dose (1).

Ethene showed for photons a large initial recombination (1). It might have been expected that the ion recombination for neutrons in ethene would result in a very small reading, thus providing a neutron insensitive detector. However, the readings for 2.1 MeV neutrons with ethene up to about 1.5 MPa indicated a considerable neutron sensitivity.

Conclusions

- The use of methane in the TE high-pressure chamber appears to provide the most suitable detector among the investigated gas-chamber combinations for total dose determination in neutron photon fields for neutron energies from 0.9 to 15 MeV.
- The pressure dependence of the relative reading of the TE and Al chamber filled with CH_4 can be used to assess neutron energy and cavity size effects.
- Ion recombination in CH_4 can be used to assess neutron energy.
- The use of Ar in the Al high-pressure chamber seems most suitable to determine the foton dose, as an alternative method to GM-counters.

Reference

Zoetelief, J., Engels, A.C., Bouts, C.J., Broerse, J.J. and Hennen, L.A. In: Proc. Fifth Symp. on Neutron Dosimetry. Commission of the European Communities, EUR-9762, Vol. II. pp. 705-715 (1985).

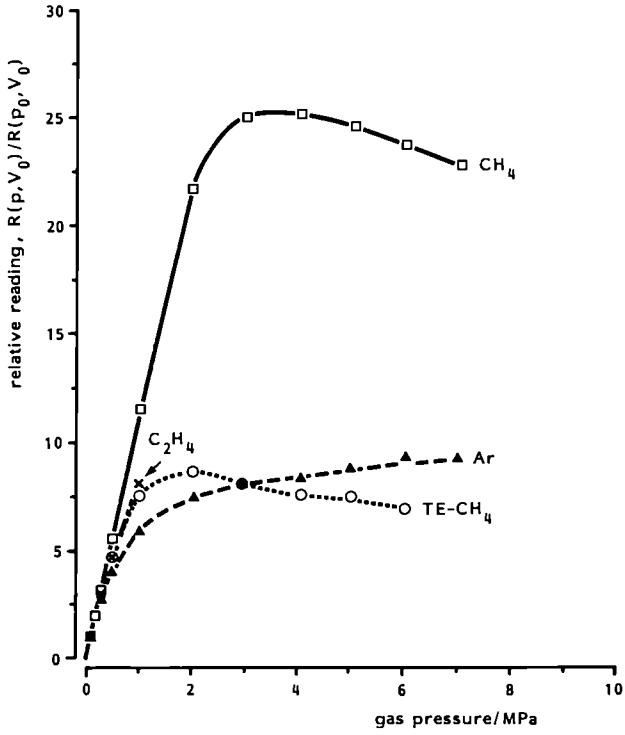


Figure 1. Relevative reading of the TE-high-oressure chamber as a function of the pressure of various gases for 2.1 MeV neutrons.

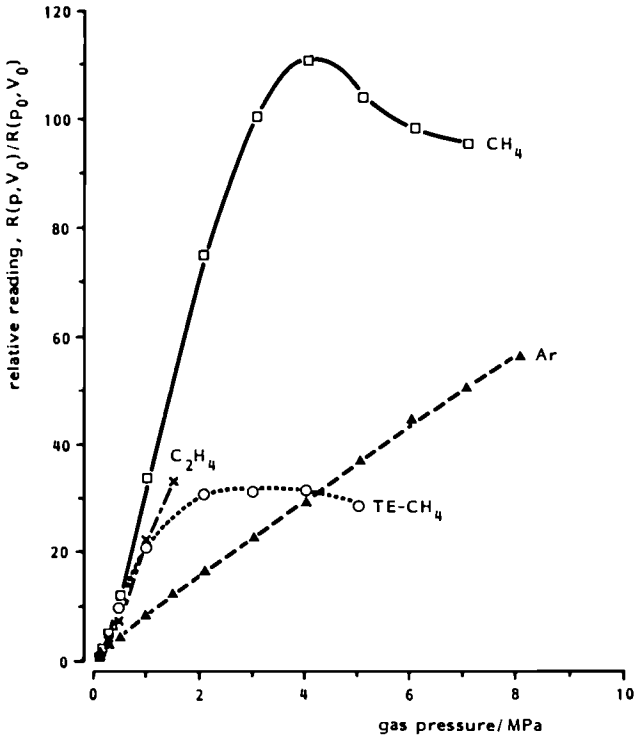


Figure 2. Relative reading of the Al high-pressure chamber as a function of the pressure of various gases for 2.1 MeV neutrons.

IV. Objectives for the next reporting period

The experimental studies with the TE and Al high pressure ionization chambers employing CH_4 Ar, TE-gas and C_2H_4 will be 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 will be 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 studied up to now. Depending on the results obtained at 0.5 MeV a start will be made to investigate a practical high-pressure ionization chamber dosimetry system under actual conditions for radiation protection.

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

Dr. N. Golnik, Institute of Atomic Energy, Swierk, Poland.
Dr. J.J. Coyne, National Bureau of Standards, Gaithersburg, MD, USA.

VI. Publications:

Zoetelief, J., Golnik, N. and Broerse, J.J. Studies of high pressure ionziation chambers in neutron and photon fields. Proc.Sixth Symp. on Neutron Dosimetry (to be published in Radiation Protection Dosimetry).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

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

Contract no : B16-A-006-UF

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

Prof. K.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 R G Chambers

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:

The large tissue sample held remains rich in scientific data especially in relation to particles retained at the visceral pleura. The latter is considered important and the in depth investigation will continue in 1987.

III. Progress Achieved:

Work has continued on a study of alpha-emitting particles retained at the visceral pleura. This, however, has been limited and the data obtained has not substantially added to the information given in our last report. Within the funding provided, the largest effort during 1987 has been concentrated on studying alpha-emitting particles in human bone 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 1988 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:

316-006-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 R G Chambers

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:

As tissue collected during 1986 reaches the minimum recommended storage period of one year, analysis of the autoradiographs can commence. It is expected that the year will be dominated by routine analysis of the sample set. It is hoped that by the end of 1987 a cross section of data on alpha-activity levels in autopsy bone from natural background sources will emerge. One particular aim will be to set limits to the presence in bone of naturally occurring α-radionuclides.

III. Progress achieved:

Methodology. During 1987 further bone samples were collected. We now have a sizeable sample set from over 120 cases. The majority of cases comprise samples of rib, vertebra and skull but the set includes 8 whole femurs and 2 vertebral columns. All of this tissue has been mounted against CR-39.

We report below our analysis carried out on a set of bones from adults and children which were obtained by the National Radiological Protection Board and came mainly from residents in West Cumbria. In addition to our own measurements the NRPB have determined plutonium levels separately by radiochemical analysis. The breakdown of cases is as follows:

Adults

Case	Age/Sex	Residence
N1	64M	Oxfordshire
N2	82F	West Cumbria
N3	65F	"
N4	22M	"

Children

Case	Age	Residence
X7	14	Newcastle
X8A	15	West Cumbria
X8B	15	"
X9	9	"
X10	<1	"
X11	Still Born	"
X12	4	"

The methods of analysis using CR-39 autoradiographs have been described in our previous report and are the subject of a recent publication (see publication). It is important to note that the tissue is mounted wet and unfixed. This is to avoid the possibility of contamination at the low levels of activity present.

Results. During the year significant progress has been made in our understanding of alpha-emitters in bone at natural levels of exposure. One important feature is that the activity is dominated by ^{210}Po. (There is a marked lack of point double and triple decays characteristic of the decay of ^{222}Rn produced from the decay of ^{226}Ra and from which the activity of the latter in bone is calculated.) In our previous report we had difficulty in interpretting the α-autoradiographs because we persistantly saw activity not only in bone but also in the surrounding tissue and blood. An example is illustrated in fig. 1 taken from the vertebra of case N1. This is the same example as was shown in last year's report. In contrast we have recently published an autoradiograph of a rib section, fig. 2, which was freeze dried in a laboratory that handles plutonium. The example is shown to illustrate that the technique is well capable of producing well defined autoradiographs at low-levels of exposure and therefore that the effect in wet tissue is real.

During the year our attention was drawn to recent unpublished data showing α-autoradiographs of rat femur after administration of ^{210}Po. These show similar behaviour to that illustrated in fig. 1. As a result of this animal investigation, it is now clear that our own results do indeed show a true picture of the distribution of α-activity in human bone at natural levels of exposure. In particular, it appears that ^{210}Po cannot be described either as a bone volume or a bone surface seeking α-radionuclide. It appears to have no special affinity for bone and as such is present everywhere throughout bone and neighbouring tissue and blood and crucially in the marrow, including the red marrow. This

finding has important implications for the radiation dose to red marrow at natural levels of exposure: in particular the dose from alpha-emitters is significant in comparision with that from low LET radiation.

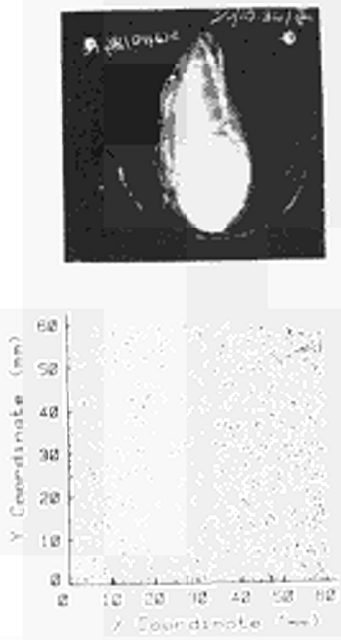


Fig. 1

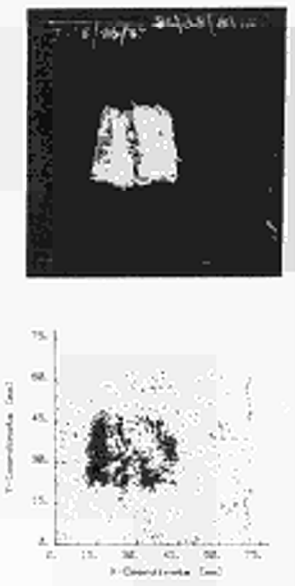


Fig. 2

Samples from a total of 22 sites in the above cases have been analysed and activity and dose values calculated for red marrow. The overall results are given below.

Summary of Measured Alpha-particle Activities and Derived Doses in a set of Bones from UK Adults and Children.

1. Activity Values in Bq.kg^{-1}

	Total	^{226}Ra	Pu*
Adult's Bones	1.66 +/- 0.03	0.22 +/- 0.03	<0.010
Children's Bones	0.39 +/- 0.01	0.13 +/- 0.03	<0.002
Children's Liver	0.13 +/- 0.02	0.04 +/- 0.01	-

*Note that the Pu values were measured separately at NRPB; see ref 1.

2. Ratio ^{226}Ra/Total Activity

Adults: 0.13 +/- 0.02; Children: 0.33 +/- 0.07

3. Dose Values

		$\mu Gy.y^{-1}$	$\mu Sv.y^{-1}$
Adult's Bones	Total (Mainly ^{210}Po)	44	986
Childrens's Bones	Total (Mainly ^{210}Po)	10.4	208
Black Report[2]	^{210}Po	0.23	5.5
	^{226}Ra	0.65	13
	^{210}Pb (High LET)	1.45	29
	Total High LET	2.38	47.5

Note that in this table dose values for ^{226}Ra have not been calculated. Final plastic calibration for ^{226}Ra and conversion to marrow dose is still in progress but since the activity in bone is clearly dominated by ^{210}Po, the total dose values to red marrow quoted will not change significantly.

Discussion

The direct measurement of alpha-particle activity in red marrow has shown that α-radiation contributes significantly to the total radiation dose received. This is dominated by ^{210}Po with ^{226}Ra having a much smaller contribution to the red marrow dose at natural levels. Furthermore the dose marrow dose from plutonium in these non-occupationally exposed cases represents an extremely small component of the total α-dose. The low LET dose to red marrow has been calculated as 1 $mSv.y^{-1}$ (see ref 2). Our present measurements show that in the samples analysed, the high LET dose in adults is 0.89 $mSv.y^{-1}$ and in children 0.20 $mSv.y^{-1}$. Further work is in progress to obtain better statistics in children as a function of age.

Acknowledgements

We are very grateful to our colleagues at both the MRC Radiobiology Unit and NRPB, Harwell for helpful discussions and advice in this work. We thank Dr Don Popplewell at NRPB for providing the results of his plutonium measurements and Dr Nic Priest for providing his unpublished data on ^{210}Po in rat bone.

References

1. Popplewell D. S., Ham G. T., Dodd N. J. and Shuttler S. D. "Plutonium and ^{137}Cs in Autopsy Tissues in Great Britain", Science of the Total Environment Vol 70 pp321-334 (1988).
2. "Investigation of the Possible Increased Incidence of Cancer in West Cumbria" Report of the Independent Advisory Group, Chairman: Sir Douglas Black, HMSO, ISBN 0 11 321006X (1984).

IV Objectives for the next 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.

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

VI Publications.

Tatzialekou M., Henshaw D. L. and Fews A. P. "Automated Image Analysis of Alpha-particle Autoradiographs of Human Bone" Nucl Instr Meths A263, 504-514 (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 1987, since no litigation cases became available for analysis. We have, however, analysed a set of bone samples from adults and children from natural background cases where we have used CR-39 to look at total α-activity and NRPB have measured plutonium levels using radiochemical analysis. These results are the subject to a separate report.

The lack of litigation cases for analysis and lack of adequate funding have combined to to make the contruction of purpose-built facilities in the Bristol Laboratory unfeasible at the moment.

The CEC funding provided for 1987 has instead been used principally on our studies in human bone for which a full report has been made.

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. If adequate funding becomes available, proposals will be drawn up for new laboratory facilities for this work.

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

1987

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-0C4-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, Dr. G. Bertoncello

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 tumor 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 tumor 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 tumor induction in mice irradiated with low doses of neutrons and X rays.

Initiation of the follow-up of mice irradiated with fractionated doses of fission neutrons.

Additional consideration of the risk of neoplastic transformation per cell in epithelial tissues.

III Progress achieved:

The main activity in 1987 was directed to the continuation of in vivo studies of the dose-response relationships for late somatic effects induced by either sparsely or densely ionizing radiation in mice. In particular, the attention was focused upon tumor induction and life-span shortening in female mice at low doses of fast neutrons and X rays. These studies were fostered by previous experimental observations, indicating a significant excess risk of solid tumors in male mice at fission neutron doses around 0.1 Gy, while X-ray doses below 3 Gy appeared to be little effective in respect to this endpoint.

Complete observation of survival and late pathology was carried out on over 2000 $BC3F_1$ female mice, which had been irradiated with single doses of either 1.5 MeV neutrons, produced by a Van de Graaff accelerator (0.5 to 16 cGy), or 250 kVp X rays (HVL=1.5 mm Cu) (4 to 256 cGy), and followed until spontaneous death.

Data of mean survival time appear to be consistent with a linear decrease with the dose, without threshold, for both fast neutrons and X rays, although the effect was too small to be significant at the lower doses, i.e., below 8 cGy and 64 cGy, respectively. The ratio of the fitted linear slopes was 12.3 ± 1.8.

Mortality rates for specific causes of death were examined using a Weibull-type time dependence, and the relative risks at the various doses were calculated accordingly. This calculation indicated that the risk of death with ovarian tumors is already appreciably increased below 0.1 Gy for both neutrons and X rays. For other solid tumors the minimum doses of this experiment producing a significant increase of the risk are 8 cGy and 64 cGy, respectively.

These findings were consistent with the analysis of the final tumor age-adjusted incidence (Fig. 1), which showed that for ovarian tumors the dose-response curve presents a steep rise starting between 5 and 10 cGy for both neutrons and X rays. For other solid tumors, the incidence was significantly increased above that in the control, starting from the doses of 8 cGy and 128 cGy, respectively. This was also confirmed by the results of a statistical analysis, testing the existence of a positive trend of tumor incidence with the dose.

Research activity in 1987 also comprised the continuation of the experimental in vivo study of the carcinogenic effect of fractionated doses

of fission-neutrons and X rays, in particular the follow-up of the animals irradiated during the previous year.

The results of experiments aimed at studying the risk of neoplastic transformation of epithelial cells have been discussed and the feasibility of new experimental series has been contemplated.

Further experimental work was also carried out during this year to study the dependence on the damaging agent (radiation and/or chemicals) of the relative yield of different types of chromosomal aberrations in human lymphocytes.

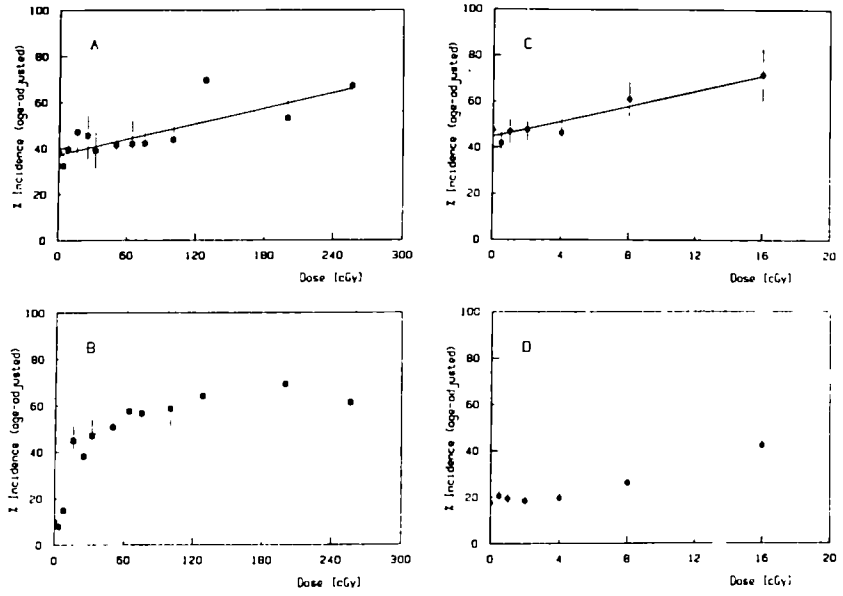


Fig. 1. Percentage incidences of solid tumors induced in mice by X rays (A) and fast neutrons (C). Plots B and D are for ovarian tumor induction after X-ray and neutron irradiation, respectively.

IV Objectives for the next reporting period.

Analysis of data of tumor 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.

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

VI Publications:

1) Di Majo, V., Coppola, M., Rebessi, S., Bassani, B., Alati, T., Saran, A., Bangrazi, C. , Covelli, V. Radiation hepatocyte survival and liver tumor induction in the mouse. Int. J. Radiat. Biol. 51, 749, 1987.

2) Coppola, M., Bertoncello, G. Neutron RBE at low doses: micronuclei formation in the murine eye-lens epithelium. Int. J. Radiat. Biol. 51, 914-915, 1987.

3) Di Majo, V., Coppola, M., Rebessi, S., Saran, A., Alati, T., Covelli, V. Radiation epithelial cell survival and tumor induction in the mouse. In: Proceedings of the 8th International Congress of Radiation Research, Edinburg (Fielden, E.M., Fowler, J.F., Hendry, J.H., Scott, D., eds.), vol. 1, p. 193, Taylor & Francis, 1987.

4) Covelli, V., Coppola, M., Di Majo, V., Bassani, B., Rebessi, S. Dose-response curves for carcinogenesis at low doses of neutrons. In: Proceedings of the 8th International Congress of Radiation Research, Edinburg (Fielden, E.M., Fowler, J.F., Hendry, J.H., Scott, D., eds.), vol. 1, p. 208, Taylor & Francis, 1987.

5) Covelli, V., Coppola, M., Di Majo, V., Rebessi, S., Bassani, B. Relazione dose-effetto per l'induzione di tumori dell'ovaio del topo alle basse dosi. In: Abstracts of IV Convegno Nazionale SIRR, S. Teresa, Lerici, p. 29, 1987.

6) Di Majo, V., Coppola, M., Rebessi, S., Covelli, V. Efficacia biologica relativa per effetti somatici tardivi nel topo. In: Abstracts of IV Convegno Nazionale SIRR, S. Teresa, Lerici, p. 30, 1987.

7) Covelli, V., Coppola, M., Di Majo, V. Rischio di trasformazione neoplastica a livello cellulare. In: Radiazioni e Tumori, Atti VIII Congresso Nazionale AIRM, Ischia, 1987 (in press).

8) Coppola, M., Covelli, V., Di Majo, V., Rebessi, S. Study of dose-response relationships for late somatic effects of low neutron doses. Proceedings of the 6th Symposium on Neutron Dosimetry. Radiation Protection Dosimetry (in press).

9) Covelli, V., Coppola, M., Di Majo, V., Rebessi, S., Bassani, B. Tumor induction and life shortening in $BC3F_1$ female mice at low doses of fast neutrons and X rays. Radiat. Res. (in press).

10) Coppola, M. Aspetti radiobiologici rilevanti ai fini della radioprotezione. Sicurezza e Protezione, ENEA/DISP (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-A-193-I

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

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

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Via Romea 4
I-35020 Legnaro (Padova)

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 diameters 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 proportional counter had to be manufactured and the anode and grid characteristics optimized in order to reach the best performances at low pressure.

III Progress achieved:

Because of the severe delay in the signature of the contract (the Istituto Nazionale di Fisica Nucleare has signed only in the autunm of 1987) the experimental work in the last year could not be developed following the objectives of the project. Nevertheless the work to provide the Legnaro Laboratories of a facility to produce collimated neutron beams has been carried on. Reproducible and dose-calibrated neutron beams are necessary to study the quality changes when different simulated diameter are used.

IV. Objectives for the next reporting period:

An effort will be done in order to recover the delay. In particular the experimental work will be devoted to the study of the working characteristics of a TEPC, with and without field-grid, at simulated diameter less than 1 um.

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, G. Tornielli
"Fasci collimati di neutroni presso i Laboratori Nazionali di Legnaro"
IV Convegno Nazionale della Società Italiana per le ricerche sulle radiazioni (SIRR)
S. Teresa, Lerici 15-16 settembre 1987.

P. Colautti, G. Talpo, G. Tornielli
"A Facility to Produce Collimated Neutron Beams at the Legnaro Laboratories"
Proceedings of the IV Symposium of Neutron Dosimetry, Neuherberg, October 12-16, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no: BI6-A-192-F

Université de Limoges
Allée André Maurois
F-87060 Limoges Cédex

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

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Dr. J.C. Vareille
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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. VAPEILLE

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 developed dosemeter.

II. Objectives for the reporting period:

* Etude de la contribution des atomes lourds de recul (C et O dans notre cas) à la réponse du dosimètre.
* Premières vérifications expérimentales du système $(CH_2)_n$-CR 39 conçu d'après les calculs rapportées dans la période 1986 et les résultats précédents sur la contribution C et O.
* Premiers essais d'implantation de ^{10}B pour obtenir un convertisseur aux neutrons thermiques de sensibilité voisine de celle des neutrons rapides.

III Progress achieved

1 - Methodology

* Les ions C et O générés dans le radiateur ont une contribution négligeable à la réponse globale, compte-tenu de l'absorption qu'ils subissent dans le radiateur lui-même et de leur faible énergie (énergie neutrons inférieure à 3,4 MeV). Par contre, ceux générés dans le détecteur conduisant à des traces qui persistent au-delà du parcours des ions (propriété signalée du CR 39) sont en prendre en compte.

Grâce à l'accélérateur HVEE 400 kV de notre laboratoire nous avons défini expérimentalement les limites d'enregistrement des ions C et O allant de 40 keV à 700 keV. Ces limitations sont appliquées aux distributions énergétique et angulaire, que nous calculons, des ions C et O générés dans le CR 39.

* Les vérifications expérimentales se font en utilisant la méthode différentielle qui permet de s'affranchir de la réponse C et O et du bruit de fond.

* L'implantation sur l'accélérateur HVEE est effectuée dans du polyéthylène et dans des feuilles métalliques, avec des fluences variant de 10^{14} à $2\ 10^{15}$ atomes cm^{-2}.

2 - Results

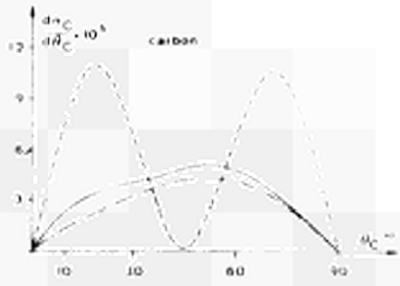

Fig. 1 : Distribution angulaire des Carbones de recul

[illegible]

* La figure n° 1 illustre la distribution angulaire des ions carbone générés dans le CR 39, dans le cas de trois énergies. Le problème de l'enregistrement des ions implique la connaissance de leur distribution angulaire (limitation θ_L) mais aussi énergétique (θ_L dépend de l'énergie). On calcule ces deux distributions et on leur applique les conditions d'enregistrement de C et O. On constate dans le tableau n° 1 que les prévisions théoriques sont plus proches des résultats expérimentaux.

Tableau 1

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Par exemple, a 1 MeV, le calcul donne N_{Td} = 756 traces cm^{-2} alors que l'experience conduit a N_e = 780 traces cm^{-2} ; rappelons que si les C et O n'étaient pas pris en compte, le calcul ne donnerait que N_{pd} = 300.

* En utilisant la méthode différentielle on compare l'efficacité effective E_e (traces mSv^{-1} cm^{-2}) calculée par le programme exposé dans le précédent rapport et les résultats expérimentaux obtenus dans le cadre des irradiations communes du CENDOS, groupe n° 5, et à Fontenay-aux-Roses (3, 4 MeV) (figure n° 2). A noter que les mesures sont faites avec un CR 39 de provenance PERSHORE et un autre détecteur du même type, appelé CAD, fourni par la Société ESSILOR. On note l'excellente concordance entre ces deux détecteurs (figure n° 2).

* Les ions ^{10}B implantés dans le convertisseur ont des énergies faibles (50 keV ou 80 keV) de façon que la profondeur d'implantation exclut une absorption des α emis dans le radiateur. Dans ces conditions, on obtient une efficacité effective qui augmente avec la densité d'implantation (figure n° 3).

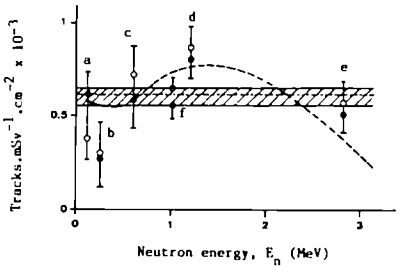


Fig 2 · Efficacité effective --- théorie
● CAD - O CR 39

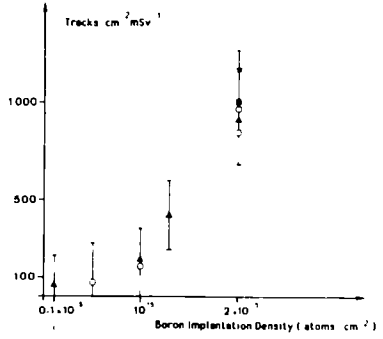


Fig 3 Efficacité dosimétrique avec radiateur implanté: □ * ◻ $(CH_2)_n$/Δ métal

3 - Discussion

On note que la contribution des ions C et O est importante dans la réponse d'un système dosimétrique. Bien sûr on a représenté le cas du CR 39 seul, mais lorsque un convertisseur existe le problème se pose encore de façon non négligeable. Ainsi avec le convertisseur de polyéthylène optimisé (cf. C.R. activité 1986) d'épaisseur e = 35 µm, la contribution des C et O est de l'ordre de 45 % de la reponse totale a des energies de 2,8 MeV ou 3,4 MeV. De plus, dans le cas de convertisseur plus épais (équilibre protonique) aux mêmes énergies, la réponse en C et O demeure 20 % de celle correspondant aux protons enregistrés issus du radiateur. Si on souhaite relier les calculs à l'expérience, cette contribution complique le travail et on a intérêt à utiliser la méthode différentielle.

Pour ce qui concerne la réponse aux neutrons thermiques, l'implantation voisine de 2 10^{15} atomes de Bore cm^{-2}, conduit à une valeur de E_e semblable à celle obtenue aux neutrons rapides. Cela est encourageant pour aborder le test complet du dosimètre aux neutrons thermiques - rapides.. L'implantation dans des feuilles métalliques sera aussi utilisée. Bien sûr le comportement de la réponse en fonction de la densité d'implantation mérite de nouvelles investigations

IV Objectives for the next reporting period:

La phase de test systématique qui peut ouvrir la voie à la faisabilité du dispositif nécessite impérativement un système de dépouillement automatique. L'appareillage livré en décembre 1987 sera donc testé et calibré avant d'aborder toute série de mesures. La fiabilité du dosimètre implique également de maitriser le bruit de fond que nous allons étudier de façon systématique dans le CR 39 et le CAD.
Ce travail préparatoire devrait permettre d'aborder les irradiations communes du CENDOS dans le but de tester le dosimètre $(CH_2)_n$ dopé ^{Cº} 39 ou ^{Fe} dopé $(CH_2)_n$ CAD dans la structure différentielle.

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

- Irradiations en neutrons dans le cadre du CENDOS-EURADOS (n° 5)
- Collaboration avec le SIDR - CEA - Fontenay-aux Roses.

VI. Publications:

1 -
L. MAKOVICKA, J.L. DECOSSAS and J.C. VAREILLE
Experimental study of the dosimetric efficiency of a radiator-CR 39 fast neutron dosemeter
Rad. Prot. Dos., 20, 1/2, 63-66, 1987.

L. MAKOVICKA, B. BARELAUD, J.L. DECOSSAS and J.C. VAREILLE
Detection of the thermal neutrons by CR 39 using a boron implanted convecter"
6th Symposium on Neutron Dosimetry
Neuherberg - R.F.A. - October 12-16, 1987.

2 -
L. MAKOVICKA, Thèse d'état n° 17-87, Limoges, 1987
"Contribution à la dosimetrie neutron gamma
Etude d'un ensemble radiateur-détecteur type CR 39".

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-A-005-F

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

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

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

Telephone number: 76-88.44.00

Title of the research contract:

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

List of projects:

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

Title of the project

Study on 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, A. Marchetto, M. Delahaie, P. Jaubert.

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

The reference detector, in a beta irradiation field calibrated by PTB consists of an EIC1-FWT extrapolation chamber, connected to a Keithley electrometer and an on line APPLE II computer. This study starts again after a temporary break - Simultaneously, we have brought into operation the detecting system for thermally stimulated exoelectron emission and a beta irradiation facility for sources calibrated by LMRI (France).

III - Progress archieved

Extrapolation chambers

Two ionisation chambers, type FWT-EIC1, were used. Their entrance window is a graphite coated mylar foil, 0,83 mg.cm^{-2} thick, or a 7 mg.cm^{-2} A 150 TE material absorber. The cavity of the chamber can be filled with air or methane based tissue equivalent gaz (GET) in circulation.

They are connected to a Keithley electrometer (model 642 with a measuring limit of 10^{-17} A) and an on line APPLE II computer carrying out the data acquisition and the data processing. These chambers, in electronic equilibrium conditions, were irradiated in ^{60}Co beams so as to compute the areas of the collecting volume with a relative uncertainty of 2 %.

This determination has been then confirmed in a (Sr + Y)^{90} irradiation beam of a Büchler facility calibrated by PTB, at three source-detector distances : 11 cm, 30 cm, 50 cm.

The response R of a chamber can be given by the following relationship :

$$R = \frac{(\dot{D}_t)_m}{(\dot{D}_t)_r}$$

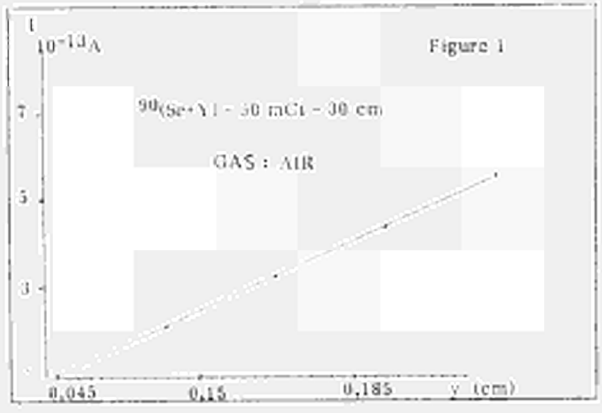
- $\dot{D}_{tm}$ is the absorbed dose rate measured with the FWT chamber, and a 7 $mg.cm^{-2}$ thick ET entrance window.

- $\dot{D}_{tr}$ is the reference absorbed dose rate in tissue, at a 7 $mg.cm^{-2}$ depth under the skin surface, given by PTB. The values of R for one detector, are gathered in table 1 :

Table 1 : Values of R ± ΔR

d_{cm}	R ± ΔR
11	1,018 ± 0,042
30	0,992 ± 0,043
50	0,999 ± 0,036

Figure 1 shows the results obtained in a beta irradiation field, calibrated by PTB : the ionisation current is plotted versus the apparent chamber depth.



For the same detector, and for the ^{90}(Sr + Y) source, experiments done with air and with GFT provide the product $S_{tg} \cdot W_g$.

.S_{tg} is the ratio of average mass collision stopping powers, in the case of the beta spectrum, for tissue and TE gas.

.Wg is the average energy required to produce an ion pair by beta rays in the gas.

S_{tg} Wg/e = 28,41 ± 2 % J/C

If we choose Wg/e = 29,2 ± 2 % J/C /1/

Then S_{tg} = 0,973 ± 2,9 %

Experiments have been carried out for the ^{90}(Sr + Y) radionuclide, with a cavity chamber having an entrance window made of graphite coated mylar (0,83 mg.cm^{-2} thick) or A 150 TE plastic (7 mg.cm^{-2} thick) - Their ratio gives the corresponding transmission factor T :

$$T = 1.036 \pm 1,8\ \%$$

An intercomparison for ^{204}Tl, organised by LMRI is actually in progress.

/1/ ICRU : report 31 (1979)

Exoelectron emission for dosimetry

For the readout of TSEE dosemeters, caracteristics of two monopoint counters have been studied. The parameters, which have been varied, were : high voltage, gas flow rate, amplification gain, discriminator level.

One of the counters was realized by M. Petel (CEA/FAR, IPSN/DPT), the other was developed by G. Holzapfel (PTB, Berlin).

Experiments have been run with a ^{14}C source and BeO dosemeters developed by the Professor A. Scharmann (Giessen).

Figure 2 shows dose response, for ^{90}(Sr + Y) irradiation.

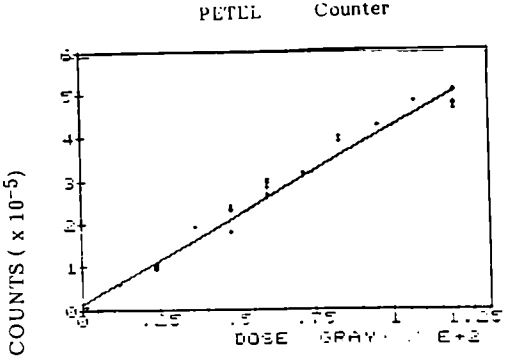


Figure 2 - Response versus absorbed dose

Irradiation facility

An irradiation facility, for beta sources 42 mm in diameter, calibrated by LMRI with filters according to ISO 6980 was developed by Fontenay-aux-Roses.

IV - Objectives for the next reporting period

The future research would be split as follows :

. Study of dosimetric characteristics, for beta irradiation, of a Lithium fluoride thermally stimulated exoelectron dosemeter, already realized by the laboratory, and comparison of the obtained results with those given by an extrapolation chamber.

. Use of dosemeters developed by other laboratories, especially for extremity dosimetry, as transfer detectors (BeO dosemeters, ultra thin TL Vinten disks).

V - Other research group

M. Petel : CEN.FAR
IPSN/DPT/SIDR
BP 6 -
92260 Fontenay-aux-Roses (France)

VI - Publications

2 - Lins Galdino Sergio : Etude comparée des caractéristiques de compteurs utilisés en émission exoélectronique thermostimulée (1987) - Rapport de stage - INT/SPR/87.1251.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-A-007-D

Kernforschungsanlage Jülich GmbH
Postfach 1913
D-5170 Jülich 1

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 prcject 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 :

J. Booz

Scientific staff :

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

B. Bednarek (Univ. Krakow), K. Morstin (Univ. Krakow)

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 at the PTB in Braunschweig
- Investigation on methods to further improve the dose equivalent response of the KFA counter
- Measurement of dose equivalent in the cold neutron facility at the research reactor of the KFA Jülich and other working areas
- Participation in the intercomparison 1987 at the PTB in Braunschweig

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 and 1986 to the Commission.

Participation in Intercomparisons at the PTB Braunschweig

During an intercomparison measurement at the accelerator facility of the PTB in Braunschweig in 1986 measurement were performed in monoenergetic neutron beams of 5 MeV, 1.2 MeV, 570 keV, 144 keV and 73 keV as well as in the fields of a ^{60}Co standard source and a heavy water moderated ^{252}Cf source.

The objectives of a detailed interpretation of the data, which was carried out during 1987, were to obtain information on the neutron energy dependance of the detectors, to study the sensitivity of the instruments and to intercompare different designs and data processing and evaluation procedures. The report of this work is in preparation and will be published as CEC-report in 1988.

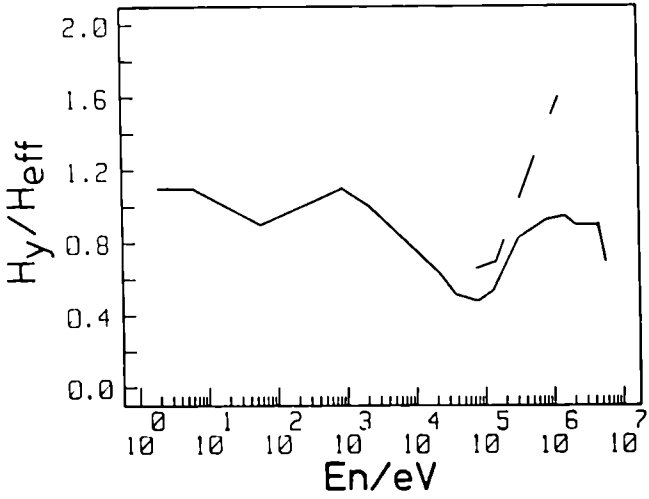


Figure 1 : Measured (dashed) and calculated (solid) dose equivalent response, H_y H_{eff}, for the KFA counter as a function of incident neutron energy E_n.

The results showed that the response of the KFA counter in terms of $H^*(10)$ is near to one for neutron energies above 1 MeV and decreases to a minimum of about 0.2 at around 100 keV. The reponse in terms of H_{eff} however, for anterior posterior irradiation is closer to one (Figure 1). The underestimation around 100 keV neutron energy will be modified to get a slight overestimation during the following report period.

The sensitivity of the KFA counter depends on radiation quality as well as on the counter construction, especially the wall thickness. In Table 1 the relative uncertainties, s_H H, with respect to ambient dose equivalent for a 30 s measurement in the considered fields are shown for a dose equivalent rate of 100 uSv h. For ^{60}Co, the dose is deposited bya great number of events with low lineal energy, y. In the neutron fields, the dose

equivalent reading is dominated by a smaller number of high y events so that the standard deviation s_H for these fields is larger than in the case of ^{60}Co.

During a second intercomparison at the PTB in Braunschweig (end 1987) experiments in monoenergetic neutron fields were performed at the accelerator and reactor facilities, using 19.2 MeV, 2.5 MeV, 570 keV, 24 keV and thermal energy neutrons. The results will be evaluated in 1988.

Table 1 : Relative uncertainty of dose-equivalent measure-ments with the KFA counter

Radiation	$S_H.H^{-1}$ %
5 MeV	22
73 keV	42
$^{252}Cf(D_2O)$	21
^{60}Co	1.3

Measurements around a Cold Neutron Facility

A new research facility, referred to as ELLA (External Neutron Guidance Laboratory), came into operation at the DIDO research reactor of the KFA-Jülich this year. Cold neutrons of 0.0025 eV are produced in a water target near the reactor core and are then guided to the experiments.

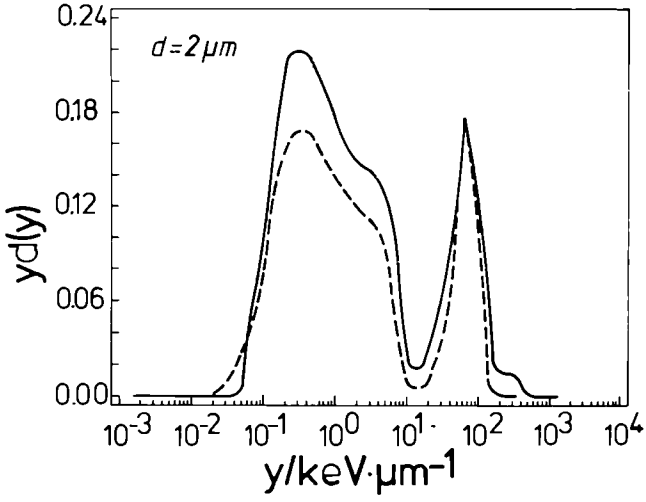


Figure 2 :

An experiment with the KFA-counter was performed in the center of the cold neutron beam, at about 30 cm in distance from the end of the beam line. The counter was placed in a beam catcher, which contains a high amount of boron. The result of this experiment is shown in Figure 2 - solid line - together with a calculation for the case that the counter is irradiated in a pure thermal neutron beam - dashed line. The calculation was normalized so that the height of the proton peak at about 100 keV.um^{-1} is equal to the corresponding peak in the experiment. If it is assumed, that the calculation models the measurement with the KFA-counter reasonably well, then it can be deduced from the comparison of the spectra that the dose distribution is indeed dominated by thermal neutrons. In addition however, there are primary photons in the beam, which can be seen from the fact that the measured photon dose component is about 20 % higher than the calculated one.

Measurements at side positions accessible to the staff showed a dose equivalent contribution of 28 % from neutrons.

IV. Objectives for the next reporting period :

- Participation in the interpretation of the intercomparison 1987 at the PTB Braunschweig

- 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 GSI Darmstadt

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

VI. Publications :

Th. Schmitz, Th. Smit, J. Booz and L.E. Feinendegen. Data Processing for Measuring Dose Equivalent with a Tissue Equivalent Proportional Counter. IAEA Seminar on the Application of Computer Technology to Radiation Protection, Bled 22.-26. Juni 1987 (in press)

J. Booz and L.E.Feinedegen. Application of Microdosimetry. Radiation Research; Proceedings of the 8th International Congress of Radiation Research, Vol 2. Taylor and Francis (London), pp. 331-337 (1987)

Th. Schmitz, K. Morstin and J. Booz. Performance of a Dose Equivalent Meter for Area Monitoring. Sixth Symposium on Neutron Dosimetry, Neuherberg 12.-16. Oktober 1987; Radiat. Prot. Dosim. 1988 (in press)

K. Morstin, A. Dydejczyk and J. Booz. Nuclear Model Calculations for High Energy Neutron Dosimetry. Sixth Symposium on Neutron Dosimetry, Neuherberg 12.-16. Oktober 1987; Radiat. Prot. Dosim. 1988 (in press)

B. Bednarek, P. Olko and J. Booz. Double Peak Effect in Proportional Counters and its Interpretation. Nucl. Instr. and Methods 1988 (in press)

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:

J. Booz

Scientific staff:

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

I. Objectives of the project:

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

II. Objectives for the reporting period:

- Evaluation of microdosimetric distributions from ^{125}I and other radiation sources to model targets of DNA
- Development of analytical functions describing energy-deposition distributions from protons and alpha particles.

III. Progress achieved:

Microdosimetric distributions from ^{125}I to model targets of DNA and cell nucleus

Calculations of lineal energy distributions were performed for ^{125}I atoms disintegrating in the center of spheres of spherical volumes simulating parts of DNA and cell nucleus. Calculation was performed in several steps as follows:

a) Monte Carlo simulation of Auger electron cascades of individual "isolated" atoms. Bookkeeping of the number of emitted electrons and photons, the accumulated multiple charge and the related potential energy.

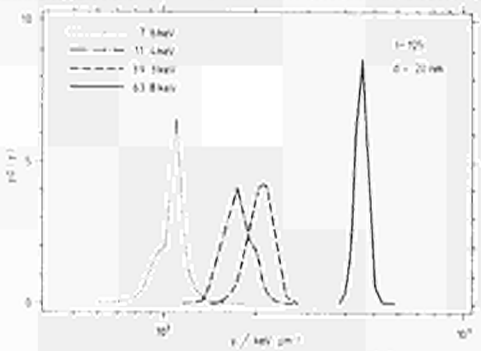
b) Calculation of the tracks in terms of ionizations from the emitted electrons in water vapour.

c) Tagging the potential energy, due to the upcharging of isolated atoms, to the point of origin of the track, i.e. the point of disintegration. This simulates the local absorption of that potential energy in solid-state matter.

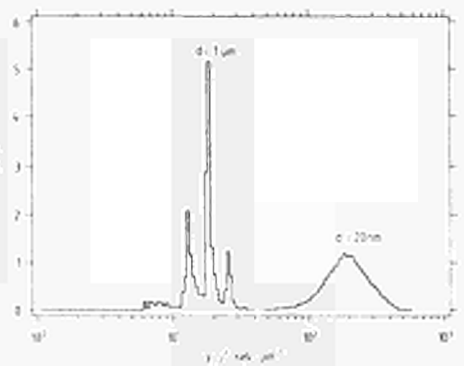
d) Evaluation of energy deposition events from the electron tracks of individual disintegrations for spherical volumes of 20 to 20,000 nm diameter and evaluation of related microdosimetric distributions d(y).

Tracks from 10,000 different disintegrations were calculated and for each disintegration at least 10 track-structure simulations were processed. All track-structure calculations were performed with the code MOCA8. Energy deposition patterns were calculated for disintegrations in the center of spheres of 20 to 20,000 nm diameter.

When considering the total energy emitted in the form of electrons, individual disintegrations of ^{125}I fall into seven distinct classes which occur with probabilities between 2.8% and 33.6% (less than 0.6 keV - 3.6%, 3.0 to 4.0 keV - 7.9%, 7.0 to 8.0 keV - 24.6%, 10 to 12 keV - 33.6%, 33 to 35 keV - 8.2%, 37 to 39 keV - 19.3%, 61 to 66 keV - 2.8%). Examples of d(y) for individual disintegrations from the four most important classes are presented in Fig. 1 for spheres of 20 nm diameter. The distributions differ with regard to the maximal lineal energy, y. All, however, are above 60 keV/µm. In other words, disintegrations of ^{125}I in DNA are always high-LET events.



Arbitrary disintegrations of ^{125}I are calculated by summing up the distributions of individual disintegrations which are weighted according to the probability of their occurance. Fig. 2 shows two examples of such dose distributions which have been derived from 10,000 different decays of ^{125}I atoms disintegrating in the center of spherical target volumes. For a volume of 20 nm diameter, a Gaussian-like distribution is obtained. For 1 µm diameter, sharp peaks made up by the different classes of disintegrations are discernable.



Energy deposition distributions for protons and alpha particles.

An analytical function was developed to simulate energy deposition spectra in spherical volumes. It is applicable for calculating the straggling of energy deposition even in DNA. Statistical fluctuations of the energy deposition by protons and alpha particles were calculated in sites of nanometer to micrometer dimension.

The calculation distinguishes between particles crossing the sites (ion events) and those passing outside and depositing energy only through secondary electrons (delta events). The straggling of energy deposition for the ion- event distribution of ionizations, $f_1^{(i)}(j)$, was expressed by a two parametric Fermi-like function. The delta-event spectra, $f_1^{(\delta)}(j)$, were approximated by a function depending on the mean value, $\bar{j}_F(\delta)$, of the distribution only. The total probabilty of producing a given number of ionizations in the site is obtained by adding, delta and ion distributions, using appropriate weighting factor.

Basing on track-structure simulations performed with the ion transport code MOCA-14, the complete set of model parameters was calculated for protons and alpha particles in the energy range 0.3 to 3.5 MeV/amu and for site diameters 1 to 1000 nm.

This analytical simulation of ionization distribution will be used to calculate biological response functions and to analyse cell response at low doses.

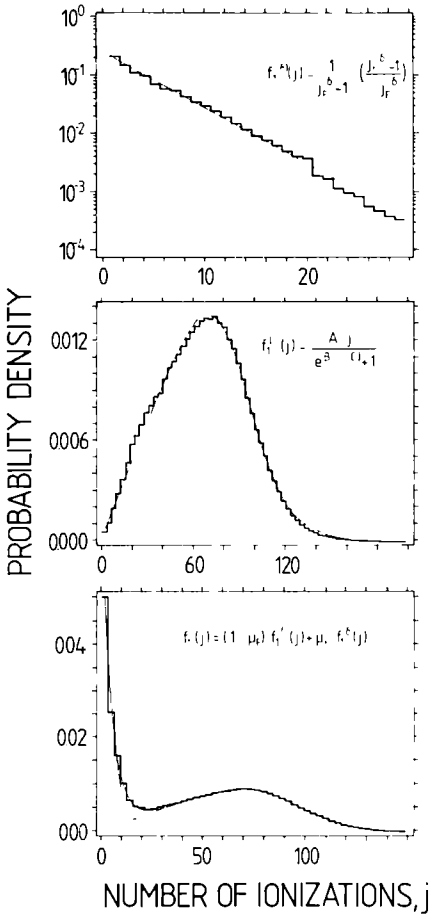


Fig.1.
Frequency distribution of ionizations produced by 1.2 MeV alpha particles in spheres 20 nm modelling a piece of DNA nucleosome fiber. Fig. a) delta-event and Fig. b) ion-event distributions of ionizations. Fig. c) shows how the total distribution of ionization is produced from the partial ion- and delta-event distributions. μ_F is the fraction of delta events.

IV Objectives for the next reporting period

- Development of analytical functions describing the ionization distribution from monoenergetic electrons in model targets of 2 to 8000 nm
  Application of these functions to the electrons emitted from ^{125}I
- Application of these functions and those developed for the description of ionization distributions from charged ions to various radiation modalities

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:

J. Booz and L. E. Feinendegen: A microdosimetric understanding of low-dose radiation effects. Int. J. Radiat. Biol. (1988) in press

I. Pomplun, J. Booz, A. Dydejczyk and L. F. Feinendegen: A microdosimetric interpretation of the radiobiological effectiveness of I-125 and the problem of quality factor. Radiat. Environ. Biophys. **26**, pp.181-188 (1987)

J. Booz, H. G. Paratzke, I. Pomplun and P. Olko: Auger-electron cascades, charge potential and microdosimetry of iodine-125. Radiat. Environ. Biophys. **26**, pp. 151-162 (1987)

D. E. Charlton F. Pomplun and J. Booz: Some Consequences of the Auger Effect: Fluorescence Yield, Charge Potential, and Energy Imparted. Radiat. Res. **111**, pp. 553 564 (1987)

I Pomplun, J. Booz and D. T. Charlton: A Monte Carlo Simulation of Auger Electron Cascades. Radiat. Res 111, pp. 533-552 (1987)

J Booz and I T. Feinendegen. Application of Microdosimetry. Proc. of the 8th ICRR, Edinburgh, Vol 2, ed. E M. Fielden et al., pp. 331 337 (1987)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor.

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

Contract no.: B16-A-019-UK

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 routines will be developed further, if possible, (a) to improve the goodness of fit to data (including the effect of temperature gradient if necessary) and (b) to reduce the computing effort required for analysis. The use of other techniques to aid the process of separating glow curves into their component glow peaks will be pursued. Experimental data on the change in glow curves with dose will be analysed fully and other data obtained previously will be analysed as time permits. Additional experiments to complete and supplement sets of data will be performed as required. A little effort will be given to the mathematical modelling of simple trap structure, and their use to demonstrate effects such as supralinearity.

III. Progress achieved:

A. Glow curve analysis

A new technique for analysing TL glow curve structure by comparing glow curves with a "standard" glow curve which has been well characterised. It improves the identification of individual peaks and gives a quantitative measure of changes in peak intensity, normalised to unit dose, for different annealing, irradiation and storage conditions. The ratio of the height at each point along a glow curve to the height of corresponding points on the "standard" glow curve is determined (each glow curve is first normalised to unit dose). The ratio curve so obtained has flat regions where individual glow peaks dominate. The value of the ratio in these regions gives the sensitivity for that glow peak compared with the same peak in the standard curve. Using these ratios and the best estimate of glow peak shapes, the technique allows successive stripping of glow peaks from glow curves, so that complex glow curves can be separated into their component peaks. The use of the glow curve ratio technique, combined with the non-linear least squares fitting technique previously developed, is proving to be a powerful tool.

The technique is currently being used to determine the supralinearity and saturation characteristics of a large number of peaks in ^{7}LiF TLD-700 (Harshaw Chemical Co) both for the normal readout and the UV phototransfer readout used for dose re-estimation. The TLDs have been irradiated with gamma rays in the dose range 0.01 Gy to 20 kGy. The technique can easily also be applied to examining the variation in the intensity of glow peaks as a result of various annealing, irradiation and storage treatments.

The non-linear least squares fitting program (written in FORTRAN 77) has been successfully transferred to a Personal Computer and other relevant programs will be transferred. This will facilitate the progress of the project.

B. Experimental artefacts

The work reported previously on variations in thermal contact and on the temperature gradient across chips of LiF has shown that these effects are usually insignificant at the heating rate of $1^{o}C\ s^{-1}$ normally used. However, occasional anomalous results are probably due to variations in thermal contact.

C. Experimental study of changes in sensitivity after high doses

We have investigated a reported progressive increase in sensitivity of lithium fluoride sintered chips (Vinten) after repeated exposures to 2 Gy of gamma radiation with annealing at $300^{o}C$ for 1 hour and $80^{o}C$ for 17 hours. The effect of repeated anneals and repeated exposures to 2 Gy on the background, the response to 20 mGy and the response to 2 Gy have been examined. Increases were observed in the background and the sensitivities which were consistent with our understanding of the behaviour of thermoluminescent lithium fluoride. We are currently

investigating the effect of using the standard anneal procedure which incorporates heating at 400°C.

D. Theoretical modelling of the TL process

The equations describing the kinetics of the localised transition model for thermoluminescence have been solved numerically. It has been found that, contrary to expectations from the simplified equations, there exist regimes of trap parameters where glow curves of non-first-order shape are obtained.

Work on numerical calculations for the more usual TL kinetics, where transport between traps and recombination centres occurs via the conduction and valence band, has continued. A single FACSIMILE program has been produced which allows the calculation of trap filling during irradiation and trap emptying during both a storage period and the subsequent read-out cycle. This program is presently being used in a study of TL fading.

IV. Objectives for the next 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.

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

None

VI. Publications:

J A Douglas, C A perks and A D .otterill. 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).

R K Bull. The creation and filling of thermoluminescence traps during irradiation, ibid, pp 459-463.

C A Perks and R K Bull. Fading of thermoluminexcence in materials exhibiting second order kinetics. (letter to the editor), Nucl. Tracks Radiat. Meas. 11(6), pp 327-328, (1986).

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:

(a) To complete development and calibration of the 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 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:

(a) To further develop Monte Carlo calculations of response functions for both H_2 and ^4He counters to include end-effects.

(b) To analyse measured response functions for H_2 counters, including comparison with Monte Carlo calculations and their incorporation into the unfolding codes, and to derive the energy calibration equations.

(c) To make comparative measurements of the neutron leakage spectrum, from the ASPIS shielding facility at the NESTOR reactor (Winfrith), with the high-sensitivity cylindrical H_2 counters and the small spherical counters (type SP2).

(d) To measure the neutron spectrum in free-air from an ^{241}Am-Be source with the ^4He counter for comparison with other measurements and calculation.

III. Progress achieved:

A. Calculation of response functions and comparison with measurements

In order to determine the neutron spectrum from the pulse-height distribution measured by the counters, it is necessary to have a matrix of their response functions. Ideally these response functions would all be determined experimentally using monoenergetic neutrons, but since this is impracticable, a Monte Carlo code has been used to calculate the response functions for both the hydrogen and helium-4 filled counters, and so provide a means of interpolation between the measured functions. The Monte Carlo code has now been developed to include end-effects in a counter.

For the cylindrical H_2 filled counters, the Monte Carlo calculations of the response functions are in good agreement with the experimentally measured functions for the counter irradiated either end-on or side-on to its axis. This agreement, which extended to include response functions both within (trapezium shaped) and above the working-range of the counter, was obtained with the measured response functions corrected for room scatter using a shadow cone.

For the ^4He counter (6 atm ^4He plus 4 atm Ar) the calculations of the response functions have been further developed to take into account the $^{40}Ar(n,\alpha)$ and $^{40}Ar(n,p)$ reactions, as well as counter end-effects. Again good agreement between the calculated and measured response functions was observed, enabling the Monte Carlo program to be used to generate the response function matrix for unfolding.

B. Analysis of measured response functions for H_2 counters

The measured response functions obtained with monoenergetic neutrons, on the previous two visits to the National Physical Laboratory, were analysed to provide both the shape of the response functions and traceable calibrations for neutron energy and fluence-rate.

Within the working energy-range of a counter the shape of the response function matrix is taken to be a trapezium. To determine the shape of the response function, a general computer fitting program FATAL was used to fit a theoretical trapezium-shaped response function, with high-energy cut-off broadened by counter resolution, to the measured pulse-height distribution corrected for room-scatter. This theoretical fit provided parameters describing the shape of the experimental response function, including the high-energy cut-off corresponding to the neutron energy calibration and the counter resolution. Since Monte Carlo calculations of the response functions were in good agreement with the measured functions, the Monte Carlo calculations provided a convenient method of both interpolating and incorporating the measured values of the slope of the response function into the unfolding program.

For neutrons with energies above the working energy-range of the counter, the response function is no longer trapezium shaped, and an empirical method was used to adjust the response functions given by the Snidow and Warren (Nucl. Inst. Meth. 51, 109, 1967) calculation used in the unfolding program. The measured over-range response

functions were incorporated into the unfolding program, using values for a "range correction factor" of 0.8 and 0.55 for side-on and end-on incidence respectively.

Energy equations, relating the neutron energy to the channel no. of pulse height analyser, were derived for all counters. The fluence calibrations showed that for side-on irradiation, the average value of the ratio of the fluences estimated for the cylindrical counter to those determined by the long counter is 1.07 ± 0.05 (SD), implying that the numbers of H_2 atoms in the counters are close to the assumed values based on the counter internal dimensions and filling pressures.

A comprehensive report describing the development and calibration of the set of four H_2 cylindrical counters has been written.

C. Measurements of neutron spectra

A visit was made to AEE Winfrith to make measurements of the neutron leakage spectrum from the ASPIS shielding facility at the NESTOR reactor. Measurements with the high-sensitivity cylindrical counters were made at different angles of incidence for comparison with measurements made at the same location with the small spherical counters (type SP2). These results will be analysed to assist in optimising the method of interpretation for the cylindrical H_2 counters. The ^{4}He counter was used to measure the neutron spectrum above 2 MeV.

The development of the ^{4}He alpha-recoil counter, to measure neutron spectra up to 15 MeV, is now complete. Measurements have been made of the neutron spectrum from an ^{241}Am-Be source in free air and the results show very good agreement with other measurements and calculations. A report on the design and calibration of the counter, the Monte Carlo code used to calculate the response functions, the unfolding codes developed, and the measurements undertaken, has been written and is in press.

Also supported by the RPR Core Programme and Eurados-Cendos, measurements were made of the neutron spectra at two positions of health physics interest inside the containment building of a Swiss PWR at Gösgen. The measurements were made with the small spherical H_2 counters (type SP2), the large cylindrical ^{4}He recoil counter to extend the measurement range up to 15 MeV and a multisphere spectrometer to determine the neutron fluence-rate below 40 keV. The neutron spectra show a steady rise in the fluence rate as the neutron energy decreases, with major contributions to the dose equivalent from two energy regions: less than 50 keV and 100-500 keV.

IV. Objectives for the next reporting period: (only to July 1988)

(i) Publication of two comprehensive Harwell reports on research and development of both high sensitivity H_2 counter system and ^4He alpha-recoil counter.

(ii) To analyse and report upon comparison of cylindrical and small spherical H_2 counter measurements of leakage spectrum from ASPIS shielding facility at the NESTOR reactor.

This project terminates in July 1988.

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

1. Continued 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, Würenlingen, Switzerland) of neutron spectra inside containment building of PWR.

3. Collaboration through Eurados-Cendos.

VI. Publications:

1. Scientific Journals

Birch, R., Delafield, H.J. and Perks, C.A., 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 (In press).

2. Reports

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

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

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 dosimeters (lower energy and dose threshold, better reproduceability and flatter dose-equivalent response), using CR-39 plastic processed by electro-chemical 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:

(i) To hold the European Workshop on the "Development of Personnel Neutron Dosimeters Based on (Proton-Sensitive) Track Etch Detectors" (see 1986 Progress Report).

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

III. Progress achieved:

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 is to commence pre-operational trials on 1 January 1988.

The results from the Joint Irradiations have been published by KfK as a Eurados-Cendos Report (number 1987-01), KfK 4305 (see publication 1).

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 have been refereed and published in a special issue of the Journal Radiation Protection Dosimetry (vol 20 Nos 1, 2) (see publication 2).

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.

This project has now terminated. A report summarising the performance characteristics and tests on the proposed CR-39 operational neutron dosemeter will be prepared early in 1988 as part of the formal approval procedure required in the UK, under separate (ie non-CEC) funding. Copies of this report will be sent to CEC when it is available.

IV. Objectives for the next reporting period:

Project terminated.

V. 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).

VI. Publications:

Reports

1. 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.

Publications in Scientific Journals

2. 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).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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: D. T. Goodhead

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

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) Scoring and tabulation of absolute frequency distributions of energy deposition in small cylindrical volumes by mono-energetic electrons (100 eV to 100 keV), mono-energetic photons (100 eV to 10 keV) and X-ray spectra (up to > 100 keV).

(2) Determination of killing of repair deficient mutant lines of V79 hamster cells by α-particles and ultrasoft X-rays, transformation of $10T\frac{1}{2}$ mouse cells by α-particles at low dose-rate and induction of aberrations in V79 cells by α-particles at low dose-rate.

III. Progress achieved:

(1) Frequency distributions of energy deposition in small targets

We have previously produced an extensive calculation and tabulation of absolute frequencies of energy deposition by segments of mono-energetic protons and α-particles in cylindrical water volumes of dimensions 1-500 nm. These were achieved by using a Norsk Data super minicomputer to superimpose randomly the target volumes on particle tracks generated by the Monte-Carlo track structure code (MOCA-14 of Wilson and Paretzke, which includes the electron code MOCA-7. We have now used MOCA-7 to calculate corresponding energy deposition distributions for the full slowing-down tracks of a selection of mono-energetic electrons of initial energies between 0.1 to 100 keV. (MOCA-7 does not include relativistic corrections and is therefore restricted to electrons of ≤100 keV). The target cylinders have diameters (d) from 1 - 100 nm and lengths varying from $d/2$ to 4d, and thereby include cylinders which simulate DNA ($d \approx 2$ nm), nucleosomes ($d \approx 10$ nm) and chromation ($d \approx 30$ nm). For efficient spatial scoring of the very large overall dimensions of the higher energy electrons a multiple-stage initial sampling procedure was applied. For lower energy electrons direct scoring was used, and the consistency of the two methods was confirmed at intermediate energies.

Energy deposition distributions in these small targets by mono-energetic or broad-spectrum X-rays can be calculated by folding together the distributions for mono-energetic electrons weighted according to the primary electron distribution produced by the X-rays. In preparation for this, distributions of Compton- and photo-electrons have been calculated for typical X-ray spectra used in biological experiments.

Energy deposition distributions for low-energy mono-energetic photons can be scored directly from Monte-Carlo electron tracks produced with probabilities proportional to the photo-absorption cross-sections and de-excitation probabilities corresponding to the atomic composition of the irradiated material. Extensive input-tables have been formulated for absorption of characteristic K X-rays of C (0.28 keV), Al (1.5 keV) and Ti (4.5 keV) in mammalian soft tissue, DNA or tissue excluding DNA. (Thus the contributions of energy deposition within a DNA-sized target cylinder can be separated into those which arise from photon-interactions either in, or out of, the target cylinder itself). Full distributions of absolute frequencies of energy deposition have been calculated in target cylinders of diameters 1 - 50 nm for each of these three mono-energetic X-rays. Since the dominant element in tissue is oxygen, the above distributions have been compared with those obtained by using a very simplified input-table containing oxygen only; the differences were small in most cases. In collaboration with E. Jones this oxygen-only approximation was used to compare the energy-deposition distributions for K characteristic X-rays of elements of $Z = 4$ to 14 and $Z = 17,20$ in a selection of target sizes. In this way it is possible to seek regions where energy deposition does not smoothly follow initial X-ray energy and to investigate their biological significance, if any.

The internal spatial structure of energy deposition events in DNA after a variety of radiations is being investigated in collaboration with D. E. Charlton. In this way it is possible to estimate the probability of production of a single or double strand break in DNA arising from an event of given energy deposition. Combining this with the distributions of absolute frequencies of energy deposition, as computed above, allows estimations of the total numbers of strand breaks produced by the radiations.

MOCA-7 has also been used to evaluate the contribution which low-energy electrons make to the total dose deposited by high-energy electrons (up to 100 keV). It was found that in irradiations with mono-energetic 100 keV electrons about 43% of the dose was deposited via low energy (0.1 - 5 keV) electrons. This is slightly greater than earlier estimates using the method of Burch (1957) and implies, as did Burch, that low energy electrons are a major contributor ($\sim$30-50%) to the dose deposited in any low-LET irradiation.

(2) Biological effectiveness of α-particles and ultrasoft X-rays

Experiments have continued to build up a body of data on the effectiveness of radiations of different qualities in inducing a variety of relevant cellular effects. These have concentrated on radiations whose track structure can be analysed in detail down to the nanometer level as described above, especially slow α-particles (3.2 MeV) and ultrasoft X-rays (0.28 and 1.5 keV), with hard (250 kVp) X-rays or γ-rays as comparative reference.

Previously in this laboratory Jones, Cox and Thacker (see Contract No. BI6-144-UK) have isolated from V79 hamster cells three stable mutant lines which are abnormally sensitive (by factors of 2.0 - 3.1) to killing by low-LET radiations (250 kVp X-rays), apparently belong to different complementation groups and show survival curves of different shapes. Their sensitivity to high-LET radiation (3.2 MeV α-particles of LET 124 keV μm^{-1}) has now been found also to be greater than that of the parent V79 cell line, but by reduced factors of 1.8 - 2.1. Nevertheless, these α-particle sensitivity factors are larger than the average (1.6) found in earlier studies with human ataxia telangiectasia fibroblasts (relative to normal human fibroblasts). These hamster data should now make it possible to distinguish between two opposing influences on the observed RBE of sensitive cells, namely the RBE of the microscopic damage but opposed by 'wasted' damage in a single track. Preliminary experiments suggest that the sensitivity factor of at least one of the V79 mutants to low-energy electron 'track-ends' (from C-K ultrasoft X-rays) is as large as it is for hard X-rays.

Conditions have been investigated for optimum irradiation of plateau phase V79 cell lines (including mutants) with α-particles at low dose-rates over periods of hours to days requiring full control of the gaseous environment and temperature. Since the required culture dishes could best be fabricated from metal (with thin Hostaphan bases) a variety of stainless steels were tested but all were found to react unfavourably with the cells or culture medium over this extended time. These problems do not apparently occur with dishes of pure titanium.

In collaboration with U.K.A.E.A., Harwell, the effects of α-particles on $10T\frac{1}{2}$ mouse cells have been investigated. Conditions were established to mimic with α-particles the neutron irradiations used by Hill et al. (1984) when they found, surprisingly, an increase in transformation frequency with decreased dose-rate of fission-spectrum neutrons. We found cell survival to be the same after irradiation with 3.2 MeV α-particles at high ($\sim$1 Gy min^{-1}) and low (5×10^{-3} Gy min^{-1}) dose-rates. Preliminary results to date show no difference in transformation frequencies after these two dose-rates of α-particles. This is in agreement with recently published α-particle results by Hieber et al. However, the serum (Nu-Serum) which we used in our preliminary experiments is substantially different from that used by Hill or Hieber.

Methods were developed to irradiate unattached mammalian cells with low-energy α-particles despite their very limited range ($\lesssim$ 20 μm in water).

With these methods, in collaboration with S. Lorimore, mouse bone marrow has been irradiated with α-particles in vitro and then assayed in vivo for survival of colony forming units in spleen. Reproducible survival curves have been obtained, providing the first haemopoietic stem cell survival data for α-particles with precise dosimetry. It was recently discovered in this laboratory by Savage and Holloway that sister chromatid exchanges (SCE) can be induced by irradiation of unstimulated human lymphocytes by fast neutrons (42 MeV d-Be). A number of studies have shown that there is no such induction by X-rays. Apparently, therefore, this finding demonstrates a qualitative different in cellular effect between high- and low-LET radiations and an effective RBE which is infinite. Since this has interesting implications for the nature of the initial microscopic damage, we have carried out corresponding irradiations with α-particles of well-defined track structure. They were found to be yet more effective than the neutrons in inducing SCE.

Investigations have continued, in collaboration with Raju et al. at Los Alamos, U.S.A., on the biological effectiveness of C-K and Al-K ultrasoft X-rays. The observed RBEs confirm earlier results from our laboratory (RBE increasing with decreasing X-ray energy) and, in addition, show that the oxygen enhancement ratio decreases with decreasing energy and that the cell-cycle response is similar for all X-ray energies. The main uncertainty in biophysical interpretation of the effectiveness of ultrasoft X-rays is due to the finite thickness of the cells and the consequent need for assumptions as to the relevant dose-averaging procedures within the cell. To test these assumptions experiments have commenced with 10T½ cells which apparently are considerably thinner. Following our earlier measurements of cell killing and DNA double strand breakage in yeast by ultrasoft X-rays, in collaboration with Frankenberg et al., delayed plating (repair) experiments were continued.

IV. Objectives for the next 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 super-computer. 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.

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

D.E. Charlton, Physics Dept., Corcordia University, Canada.
D.Frankenberg, GSF, Frankfurt, W. Germany.
E.Jones, St. Bartholomew's Medical College, London, U.K.
S.Lorimore, Division of Radiation Oncogenesis, MRC Radiobiology Unit, Chilton, U.K.
H.G.Paretzke, GSF, Neuherberg, W.Germany.
M.R.Raju, Life Sciences Division, Los Alamos National Laboratory, U.S.A.
C.J.Roberts, U.K.A.E.A. Harwell Laboratories, Didcot, U.K.
W.E.Wilson, Radiological Physics, Battelle Pacific Northwest Laboratories, U.S.A.

VI. Publications:

Full Papers

(1) D.E.Charlton, "Atomic data required for the calculation of local energy deposition near isotopes decaying by electron capture or internal conversion." In: Nuclear and Atomic Data for Radiotherapy and Related Radiobiology, Proceedings of an Advisory Group Meeting, Rijswijk, Sept. 1985. (IAEA, Vienna), pp. 27-35 (1987).

(2) D.T.Goodhead, "Physical basis for biological effect." Ibid. pp. 37-53 (1987).

(3) D.T.Goodhead, "Relationship of microdosimetric techniques to applications in biological systems." In: Dosimetry of Ionizing Radiations, Vol. 2, eds. K.R.Kase, B.E.Bjarngard and F.H.Attix (Academic Press, New York), pp. 1-89 (1987).

(4) C.J.Roberts and D.T.Goodhead, "The effect of ^{238}Pu α-particles on the mouse fibroblast cell line C3H 10T½: Characterization of source and RBE for cell survival." Int. J. Radiat. Biol., 52, 871-882 (1987).

(5) M.R.Raju, S.G.Carpenter, J.J.Chmielewski, M.E.Schillaci, M.E.Wilder, J.P.Freyer, N.F.Johnson, P.L.Schor, R.J.Sebring and D.T.Goodhead, "Radiobiology of ultrasoft X-rays: I. Cultured hamster cells (V79)." Radiat. Res. 110, 396-412 (1987).

(6 D.T.G dhead, "Bi physical models f radiation action." In: Radiation Research, Proceedings of the 8th Int. Congr. of Radiation Research, Edinburgh, July 1987 (Taylor & Francis, London), Vol. 2, pp. 306-311 (1987).

(7) Z.S.Aghamohammadi, D.T.Goodhead and J.R.K.Savage, "Induction of sister chromatid exchanges (SCE) in G_0 lymphocytes by plutonium-238 α-particles." Int. J. Radiat. Biol. (in press).

Sh rt C mmunications

(8) M.R.Raju, S.Carpenter, M.Cornforth, M.Schillaci and D.T.Goodhead, "Cell killing by ultrasoft X-rays." In: Abstracts of Papers for Thirty-fifth Annual Meeting of the Radiation Research Society, Atlanta, Feb. 1987. (Radiat. Res. Society, Philadelphia), p. 31.

(9) H.Nikjoo and D.T.Go dhead, "Physical and biological consequences of high- and low-LET radiations in biological targets." In: Radiation Research, Proc. 8th Int. Congr. of Radiation Research, Edinburgh, July 1987 (Taylor & Francis, London), Vol. 1, p.80 (1987).

(10) H.Nikjoo and D.T.Goodhead, "Energy deposition in small size targets by high and low LET radiations." Ibid., Vol. 1, p. 87 (1987).

(11) C.J.Roberts, D.T.Goodhead, G.R.Morgan and P.D.Holt, "Transformation of C3H 10T½ with ^{238}Pu alpha particles." Ibid., Vol. 1, p. 182 (1987).

(12) J.S.Bedford and D.T.Goodhead, "Breakage of human interphase chromosomes by alpha particles." Ibid., Vol. 1, p. 226 (1987).

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: D. T. Goodhead

Scientific staff: D. T. Goodhead
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) To obtain quantitative information on production and characterization of K-characteristic X-rays by proton bombardment of selected elements from Z=5 to about Z=30, and to evaluate these in terms of usefulness as practical beams for measurement of radiation effects.

(2) To obtain accurate measurements of external and internal dimensions of cultured mammalian cells under irradiation conditions.

III. Progress achieved:

Characterisation of ultrasoft X-rays produced by proton-bombardment of solid targets has been carried out with proportional counters in vacuo as well as with attenuation and ionization measurements in air. The most detailed measurements have been carried out on targets of C and Al, the K-shell X-rays of which have been used in numerous radiobiological experiments with proton bombardment at energies of 600 to 700 keV on our Cockcroft-Walton accelerator and appropriate filtration to extract the X-ray beam from vacuum to air and to eliminate scattered protons if necessary. We have collaborated with Jones and Smith in their measurements on the Van der Graaff accelerator at St. Bartholomew's College of X-ray production as a function of incident and emission angles for proton bombardment energies of 500 keV. Targets investigated were B, C, two grades of Al, Si/C, Si/N, Au (M-shell X-rays) and TiB_2. Apart from identifying production rates and optimum angles, the proportional counter measurements highlighted the contamination which can arise from carbon deposits on the target and the consequent difficulty in filtering out the C-K X-rays for lower atomic number targets. These production measurements, in conjunction with the track structure analyses of the various ultrasoft X-rays (Project 1), allow an assessment of the likely practical and theoretical usefulness of developing additional beams for radiobiological studies.

In July 1987 we organised the First International Workshop on Techniques in ultrasoft X-ray Radiobiology. This was held in Oxfordshire and was attended by personnel of all groups active in this field in the world (as far as we are aware). It was attended by more than 40 people representing at least 14 different groups, 6 countries and 4 continents. As intended, the meeting concentrated on the diverse technical problems associated with the physics and biology of the application of ultrasoft X-rays to investigative radiobiology.

The original results from our laboratory showing the biological effectiveness of ultrasoft X-rays, despite the very short range of their secondary electrons, have now been confirmed in a number of other laboratories. In particular, similar results for Al-K X-rays on hamster cells have now been obtained in 4 laboratories, for C-K X-rays on hamster cells in 2 laboratories and C-K on yeast cells in 2 laboratories. However, the major uncertainty in detailed mechanistic interpretation of these results, especially for the C-K X-rays, arises from the rapid attenuation of the X-rays through a single attached cell and the consequent need to assume the intra-cellular distribution of target material in order to calculate a relevant average dose for the cell. The hamster cells are relatively thick, typically 4-7 μm, and recent experiments with thinner cells have not revealed the simple dependence with cell thickness which might have been expected, although the problem is confounded by the thinner cells also being, coincidently, more radiosensitive even to γ-rays. It is now clearly of central importance to carry out accurage measurements of cell thickness under the exact conditions of irradiation. Various methods have been attempted with optical microscopy of living cells, including phase-contrast, bright-field with vital stain and Namarski interference contrast but none of these provide the desired accuracy. Fixation, embedding and subsequent measurement by optical or electron microscopy provide high precision but leave open the question of possible distortion by the preparation procedures. Preliminary data suggests that laser confocal microscopy on living cells may be able to resolve this question.

Consideration has been given, in collaboration with F. O'Neill, to the production of very short, high-intensity pulses of ultrasoft X-rays by laser-bombardment of Al pellets. This would allow fast time-resolved studies of early radiation processes.

IV Objectives for the next 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.

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 Laboratories, Chilton, U.K.

M. R. Raju, Life Sciences Division, Los Alamos National Laboratory, U.S.A.

A. Smith and E. Jones, St. Bartholomew's Medical College, London, U.K.

VI. Publications:

(13) M.E.Schillacci, S.G.Carpenter, M.N.Cornforth, M.R.Raju, R.J.Sebring, M.E.Wilder and D.T.Goodhead, "RBE dependence on cell thickness using ultrasoft X-rays." In: Radiation Research, Proc. 8th Int. Congr. of Radiation research, Edinburgh, July 1987 (Taylor & Francis, London), Vol. 1, p. 182 (1987). (Abstract).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

1. Experimental and theoretical investigations of the alanine dosimeter as a personal accident dosimeter in mixed field radiation.

Head(s) of 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 dosimeter as a personal accident dosimeter 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. Objectives for the reporting period:

Investigation of changes in dose meter response following irradiation with high- and low-LET radiation.

Response of the dose meter to neutrons.

Energy dependence of the dose meter response at low-LET irradiations.

Irradiation with very heavy ions and ions of very high LET.

Use of the alanine dose meter as a transfer dose meter for postal dosimetry comparison.

Due to the temporary shutdown of the tandem van der Graaff accelerator at the Niels Bohr Institute at Risø and necessary repairs of our ESR spectrometer, changes of the intended research program for 1987 were necessary.

III. Progress achieved:

The decay of the radiation-induced free radicals in alanine and thus the fading of dose meter response following irradiation have been studied. The irradiations concern: 5-20 MeV electrons, 4-18 kV_p x-rays, ^{60}Co Y-rays, 6 and 16 MeV protons, 4 MeV/u ^{16}O-ions, 15.2 MeV/u ^{40}Ca-ions and 16.5 MeV/u ^{238}U-ions. The decay kinetics after heavy charged-particle exposures have been correlated with the calculated dose distribution around the ion path and it has been shown that the fading following high-LET exposures may be calculated from the dose distribution and the fading in dose meter response after high-dose exposure to fast electrons of very high dose rate. Determination of the special fading properties after high-LET exposures are essential for the proper use of the dose meter both in general high-LET dosimetry and in radiation protection.

The decay rate of the $CH_3\dot{C}HCOOH$ radical in L-α-alanine depends on the deposited energy density and dose rate. Long-term stability of the dose meter response following low-LET exposures has been investigated by repeated measurements of dose meters exposed to 6 and 20 MeV electrons, 4 and 16 MV_p x-rays and ^{60}Co Y-rays. The dose meters exposed to ^{60}Co Y-rays showed a decrease in signal of 1.9% over a period of 4 years in samples given a dose of 100 Gy. Dose meters exposed to pulsed radiation, i.e. linear-accelerator-produced electrons and x-rays, showed a decrease in response of about 6% over 4 years independent of the dose in the dose range of 10-100 Gy. In exposures with a pulsed 10-MeV electron beam to a dose of $5 \cdot 10^5$ Gy, which is the dose for maximum obtainable response, a decay of 13% has been observed after 2000 hours.

Time stability in dose-response has been studied after exposures with high-LET beams of 15.2 MeV/u ^{40}Ca- and 16.5 MeV/u ^{238}U-ions. For low average doses, single tracks, a decay in response of 8% and 6%, respectively, in 2000 hours has been measured. For high average doses (approx. 10^3 Gy), overlapping tracks, an increase in response of 5% and 8% for ^{40}Ca-and ^{238}U-ions, respectively, has been observed with a maximum after approximately 100 hours after exposure. Thereafter, a decay of 8% and 6%, respectively, takes place after 2000 hours. These results for single track exposures show approximately the same decay as results obtained for

6-MeV protons (6%) and 4 MeV/u ^{16}O-ions (7.5%) that was published in our last year progress report; they contradict an expected larger decay following exposure with particles of the same velocity but with a higher LET. We ascribe, however, this small decay and the smaller decay for ^{238}U-ions (rather than for ^{40}Ca-ions) to the additional exposure from the heavy-ion-induced radioactivity in the sample itself.

We have tried to correlate measured radical decay for fast electron exposures to saturation doses with calculations on energy-density distributions in the track of heavy charged particles in order to predict radical decay after high-LET exposures. We calculated the fraction of energy loss in the track deposited at doses above $5 \cdot 10^5$ Gy and converted this fraction of energy into percentage radical decay by multiplication with the amount of decay observed after fast electron exposures to $5 \cdot 10^5$ Gy. We see a good agreement between experimental data and results obtained from this crude model (Table 1). It is observed from Table 1 that the decay following the ^{16}O-ion exposure is higher than for the exposure with ^{40}Ca-ions even though the average LETs for the stopping particles in the target are comparable. This is, however, due to the higher initial energy of the ^{40}Ca-ions with a following larger fraction of the total ion energy deposited at high particle velocities where the track is wide.

Table 1. Decay in 2000 hours after a dose of 10 kGy.

	Decay meas.	Decay calc.	LET_{av} $MeVcm^2g^{-1}$	$\frac{E_{lim}}{E_{tot}}$
MeV/u	%	%		%
16 protons *	3.2	3.6	38	30
6 protons	6	4.7	119	39
4 ^{16}O	7.5	7.0	$7.4 \cdot 10^3$	58
15.2 ^{40}Ca	8	6.2	$2 \cdot 10^4$	52
16.5 ^{238}U	6	7.0	$1.1 \cdot 10^5$	58

E_{lim} = track energy deposited above a dose of $5 \cdot 10^5$ Gy saturation.
* penetrating particle.

Fast neutron irradiations have been carried out with a radiotherapy beam of neutrons produced by 12.5-MeV deuterons via a Be(d,n)B-reaction in a thick berylium target. The mean fast neutron energy was 5.6 MeV and the

gamma contamination of the total neutron + gamma dose was 4%. Exposures were measured with an ionization chamber calibrated in terms of dose to tissue, and were converted into absorbed dose in water. The value of kerma, K=0.86, was calculated from tables of Bach and Caswell for a mean neutron energy of 5.6 MeV, using the elemental composition of alanine. The factor to convert dose in alanine to dose in water was taken to be unity. In order to evaluate the effectiveness of alanine relative to ^{60}Co γ-rays to pure neutron doses from measurements performed using a mixed neutron beam, we note that the total beam dose consists of 96% neutrons and 4% gamma components. Assuming additivity of the alanine signals, the signal per unit of mixed neutron beam dose is;

$$S(D_T)/D_T = E_n \cdot R_G \cdot 0.96 + R_G \cdot 0.04,$$

where R_G is the signal per unit of gamma-ray dose and E_n is the relative effectiveness of "pure" 5.6-MeV neutrons. From this a relative effectiveness $E_n = 0.62 \pm 0.03$ is obtained.

The relative effectiveness of the alanine dosimeter has also some energy dependence for low-LET radiation. X-ray exposures have been carried out using a 250-kV_p x-ray radiotherapy unit filtered to give an average x-ray energy of 90 keV. Exposures were measured in terms of roentgen in air and converted into absorbed dose in water using tables by Hubbel. An effectiveness of the alanine dosimeter to 250 kV_p x-rays relative to ^{60}Co γ-rays was measured to be $R_X/R_G = 0.83 \pm 0.04$.

An accurate determination of the relative effectiveness of alanine exposed to 15.2 MeV/u ^{40}Ca-ions, 13 MeV/u ^{74}Ge-ions, and 16.5 MeV/u ^{238}U-ions has unfortunately up to now not been possible due to unreliable dosimetry on a very unstable accelerator. As a first step in an accurate determination of dose from these ions the range has been measured using stacked 5 μm-thick radiochromic dye film samples.

Postal dosimetry comparisons between various radiation therapy centers in Finland, Poland, USA, and Denmark have been carried out using the alanine dose meters. It is possible to compare doses within an accuracy of about 2% at the 95% confidence level.

IV. Objectives for the next reporting period:

Experimental and theoretical investigations on fading of response from low-and high-LET radiations.

Establishment of dose-response data from low energy protons and α-particles.

Investigation of radiation sensitivity by deuterization of alanine.

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

VI. Publications:

J.W. Hansen, Heavy Charged Particle Dosimetry, Theory and Application. Proc. II Symp. of Radiological Physicists, 1986, Smolenice, Czechoslovakia, 1987.

K.J. Olsen and J.W. Hansen, Radical Recombination in Single Tracks of Heavy Charged Particles. Proc. 8th Int. Congress of Radiation Research, Edinburgh, Scotland, 1987.

J.W. Hansen, K.J. Olsen and M. Wille, The Alanine Radiation Detector for High and Low-LET Dosimetry. Radiat. Prot. Dos. 19, No. 1, 43-47, 1987.

M. Waligórski, J.W. Hansen and E. Byrski, Application of the Alanine Detector to Gamma-Ray, X-Ray and Fast Neutron Dosimetry. Institute of Nuclear Physics, Krakow, Poland, Report No. 1373/PL.

RADIATION PROTECTION PROGRAMME

Progress Report

1967

Contractor:

Division of Radiation Science
and Acoustics
National Physical Laboratory
Queens Road
GB Teddington, Middx TW11 OLW

Contract no.: BI6-A-003-UK

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

Dr. J.B. Hunt
Div.of Rad.Science and Acoust.
National Physical Laboratory
Queens Road
GB Teddington, Middx TW11 OLW

Telephone number: 1-943-6853

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 during November 1986, and to carry out further calibration measurements to both extend the neutron energy range to higher and lower energies and to check the consistency of the previous measurements. It was intended to finalise the response matrix for each of the sphere systems and to test it by making use of broad-range neutron sources as integral benchmark data. On the computational side, it was envisaged that the response matrix would be calculated using the discrete-ordinates transport code ANISN, and the calculations compared with the measured values. It was also proposed to test ANISN and other unfolding codes by participating in two EURADOS Committee 4 benchmark intercomparisons.

III. Progress achieved:

The NPL sphere set is made up of nine polythene moderating spheres with diameters ranging from 7.6 to 38.1 cm, and before it can be used to characterise neutron fields a necessary pre-requisite is that the response as a function of energy must be determined for each sphere. Two approaches to deriving these response functions are available - calculation and measurement. Both methods are employed in this work because (a) the reliability of the calculations is uncertain, and (b) measurements can only cover a small part of the full energy range. The degree of agreement in the region where both measurements and calculations are possible should give some indication of the reliability of the calculations as a method of interpolating in regions where measurements are impossible.

The measurement programme has now been underway since November 1986 when an intensive three week measurement period was undertaken at PTB (see 1986 Progress Report). A second measurement period of two weeks in July 1987 extended the range of calibrations down in energy to 1 keV and up to 14.8 MeV, with a number of new energies in between. Some repeat measurements were also undertaken to check the consistency of the results between the two measurement periods.

The neutron fluence was determined using either the DePangher long counter, a proton recoil proportional counter or a proton recoil telescope. Five different neutron fluence monitors were employed, allowing an assessment to be made of any variations in the incident particle beam energy, the angular distribution of the emitted neutrons, the stability of the target material, and the stability of the incident beam current. The shadow cone technique was used to determine the room and air inscatter contributions to both the spheres being calibrated and the neutron fluence measuring device. Corrections were also applied for dead-time effects, air attenuation or outscatter, noise, photon contamination and scattering by the spheres and the neutron fluence devices into the monitors. The analysis of the first set of data has been completed except for a small correction to account for low energy neutrons scattered by the target assembly. The analysis of the second set of data is still in progress.

The preliminary results were presented at the Sixth Symposium on Neutron Dosimetry at Neuherberg, Munich in October 1987. The NPL and PTB sphere systems employing the larger spherical ^{3}He detector are in good agreement throughout the energy range, although the PTB system is consistently 5% more sensitive. The responses of the PTB system employing the small ^{3}He detector and the GSF system based upon a LiI scintillation counter agree within ±10% throughout the energy range. The results have been compared with two recent calculations published by other groups. There are marked discrepancies between the experimental

and theoretical results in both amplitude and shape, which cannot be explained by the small differences in the density of the polyethylene. This clealy indicates that the use of a calculated response matrix normalised by a one-point-calibration factor may lead to considerable errors in interpreting multisphere data.

One vital parameter for the calculations is the ^{3}He number density within the proportional counter used as the central thermal neutron detector. Because the cross section for the $^{3}He(n,p)$ reaction is well known in the thermal region, this number density can in fact be deduced from a measurement in a known thermal fluence.

Measurements have been performed using the thermal column facility at NPL, and the results have been analysed with some care. Although it is in principle a fairly simple and straightforward experiment, the interpretation of the results is somewhat involved, and in the past has not always been performed in the necessary detailed and rigorous manner. Three proportional counters are available for use with the NPL sphere system, and the ^{3}He number densities derived for these were found to be very similar, but on average about 10% lower than the manufacturers estimates. While the thermal column was in use the opportunity was taken to measure the thermal efficiency of the smaller spheres (up to and including the 25.4 cm sphere).

Calculations of the responses are being performed with the discrete ordinates neutron transport program ANISN which has now been implemented and tested at NPL. Although there still exist disagreements of up to about 10% with calculations performed using the version of ANISN run at Harwell, the excellent agreement with various test problems provided with the program, and also with Bonner sphere calculations performed by a group at the University of Texas in the USA, indicate that the program is running correctly at NPL. No final explanation for the difference with Harwell calculations has been forthcoming, but it is possible that the two versions of the program differ in some details of the algorithms used.

The initial calculational results for the NPL spheres are in reasonable agreement with experiment, but further work is necessary in order to investigate variations with polyethylene density, and to allow in some way for void regions within the spheres, and for the external steel shell of the proportional counters.

IV. Objectives for the next reporting period:

Measurement programme: The analysis of the measurements at all eleven neutron energies will be completed, and all corrections to the results will be applied. Data obtained using radionuclide neutron sources will be used as integral data to test the derived response matrix. It is hoped to carry out some measurements under field conditions.
Computational programme: The response matrix will be computed, including an investigation of the effects of changes in the input data. The response matrices of other multisphere sets will be computed. Participation in the two EURADOS Committee 4 benchmark intercomparisons (see 1986 Progress Report) will continue.

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

Drs G Dietze, M Cosack and H Klein
Group 6.5 - Neutron Dosimetry
Physikalisch-Technische Bundesanstalt
Bundesalle 100
D-3300 Braunschweig
Federal Republic of Germany

Dr H O E Schraube
Gesellschaft fur Strahlen-und Umweltforschung mbH
Ingolstaedter Landstrasse 1
D-8042 Neuherberg, Munchen
Federal Republic of Germany

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".
Sixth Symposium on Neutron Dosimetry, Neuherberg-Munich, October 12-16, 1987. To be published in Radiation Protection Dosimetry.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

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

Contract no.: BI6-A-172-D

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

Prof. W. Jacobi
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Dr. G. Burger
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:

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. Morhart, A. Wittmann, R. Wiechell, G. Burger, H. Schraube

I. Objectives of the project:

The objective of the project are investigations on the application of the new quantities as defined in ICRU 39 in neutron radiation protection. Emphasis is laid upon generation of relevant conversion functions, performance of field analysis at various working places and of exposure analysis of personnel. The latter includes studies on personal monitoring as well as on calibration techniques.

II. Objectives for the reporting period:

1. Continuation of phantom improvements based on patient's organ reconstructions.
2. Further investigations of working places and personnel exposure in case of neutron radiation.
3. Performance of and participation in personal monitoring intercomparison studies.

III. Progress achieved:

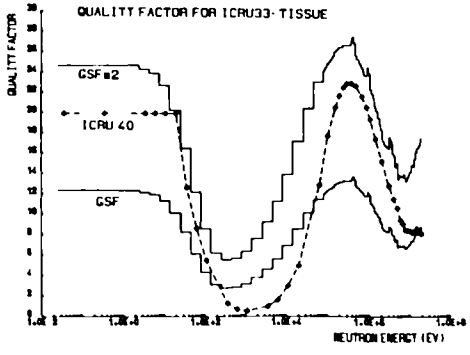
1. Three dimensional phantom reconstruction.

Further improvements have been made in the programme package MOVIE BYU providing a threedimensional surface reconstruction from serial tomographic images. A voxel model of the bodies was developed to allow for radiation transport and spatially resolved dose deposition calculations. Based on the voxel model any arbitrary section through the body, even a nonplanar one can be reconstructed and displayed. The programmme was applied to whole body tomogrammms of children (in collaboration with B 16 133 D). Relevant organs, as occuring in the definition of H_e, have been reconstructed.

2. Exposure analysis

In order further analyse theoretically exposure conditions in different working environments the necessary conversion functions were revised according to improved calculations and newer quality factor assumptions. The standard male anthropomorphic phantom was completely recalculated. Mean spectral organ fluences are now available for several exposure geometries in male and female phantoms. Conversion functions for organ dose equivalents and finally He have been derived using three different quality factors based on assumptions expressed in ICRU 16 (GSF), ICRP 51 (GSF*2) and ICRU 40 (Fig.1). The latter is based on Y rather than LET. The Y distributions have been calculated as a function of neutron energy using a revised code of J. Coyne. In addition also the operational quantities H*(10) and H'(10), as defined in the ICRU-sphere were recalculated with the new quality factors. Fig. 2 shows the ratio $H_e/H'(10)$ for three exposure geometries.

Fig. 1



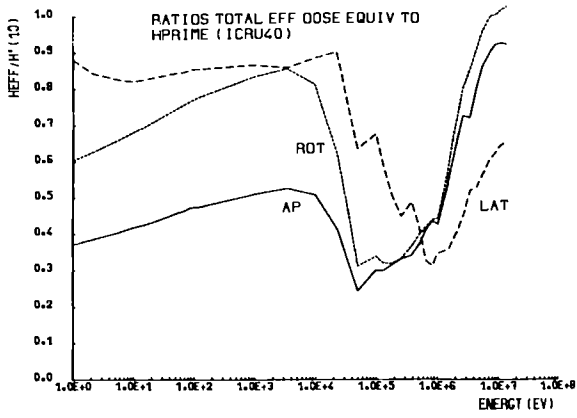


Fig. 2

The new conversion functions have been applied to 45 calculated and measured spectral distributions from calibration sources and stray and leackage fields in working environments. The ratios of H_e, (ICRU 40) to H_e, (ICRU 16) are between 1.2 and 1.6.

An experimental working place analysis was performed within three power reactors by means of multisphere measurements, the use of several commercial remcounters and two types of Albedo dosimeters (GSF, Alnor) fixed on phantoms. All instruments were calibrated at bare and moderate Cf-252 sources. Conversion functions were used for determination of MADE and ambient dose equivalent (H*(10)). Results can be summarized as follows:

- Conversion factors for MADE and ambient dose equivalent as derived from Bonner multisphere measurements were numerically very similar for all working places measured.
- Commercial remcounters overestimate the dose equivalent generally by a factor of two.
- The neutron response of the GSF Albedo dosimeter is roughly 4.5 times higher than that of the Alnor type.
- The calculation factor for the Albedo dosimeter varies by a factor of 4 for different working places. It can be reasonably determined by measurements with the 3" and 12"-sphere.

No intercomparison studies have been performed in 1987, due to the organization of the 6th Symposium on Neutron Dosimetry.

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
Dipl.Phys. Piesch, GFK-Karlsruhe
Dr. J.B. Hunt, NPL Teddington
Dr. Cosack, PTB Braunschweig
Dr. Colautti, INFN, Legnaro,
Dr. Zoeteliet, INO, Rijswijk

VI. Publications:

Burger, G., Leuthold, G., Morhart, A., Wiltmann, A.: Neutron conversion functions and their accuracy. Proc. 6th Symposium on Neutron Dosimetry, Neuherberg, 1987 (in press) (1988)

Title of the project no :

Microdosimetry and Biological Dosimetry

Head(s) of project:

G. Burger

Scientific staff:

G. Burger, G. Leuthold, M. Aubele,
K. Rodenacker, A. Voss

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. Monte Carlo simulation of charged particle tracks in water vapour have been completed for protons in the energy range from 0.2 MeV up to 15 MeV and α-particles in the energy range from 1 MeV/amu up to 15 MeV/amu. For the ions single and double differential ionization cross section given by Senger and Wilson were used. The simulation of secondary electron tracks is based on a program given by Paretzke.
The proximity function approach of Kellerer was applied to determine the contributions of ion core, isolated δ-ray and mutual interaction components to Y_D. Fig. 1 shows the results for low energy protons. As can be seen from the total Y_D the LET-aproach represented by the asymptotic values for large targets is increasingly underestimated for proton energies with decreasing energy below 500 keV energy and target sizes below 10 nm. The same holds for α-particles in the energy range below 5 MeV/amu. The reason is that 'high LET' tracks are rather homogenious with decreasing energy deposition in the penumbra. 'Low LET' tracks as represented by high energy protons show a very discontinuous local event pattern, with high event density clusters at the end of rather isolated δ-ray tracks. Y_D in these cases is grossly underestimated by the LET-approximation (see annual report 1986). Y_D was finally calculated for the proton recoil distributions from fast neutron irradiation of soft tissue.

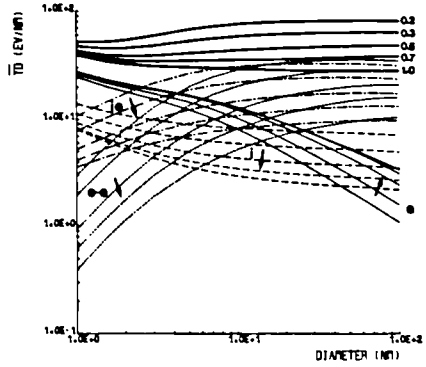


Fig 1: Individual proton track contributions to the dose mean lineal energ for proton energies of 0.2-1.0 MeV as a function of target diameter (i = ion core events only, e = isolated δ-ray events, e e = δ-ray interaction, i e = ion core-δ-ray interaction)

2. The continous revision of the code was agreed upon by J. Coyne and European collegues within EURADOS working groups I and IV. The code in the version available at GSF was applied to calculate dose mean lineal energy distributions as a function of neutron energy to determine the new quality factor, as defined in ICRU 40 (see project 1).

3. Biological dosimetry

The shape assay of mouse sperms was further investigated. To improve the material no testes biopsies were taken, but mature spermatozoa were directly collected from epididymides, alcohol fixated and Feulgen stained. In addition blood smears were taken and similarly prepared. To check the suitability of the new preparation technique and the extended feature set for morphological and DNA-chromatin texture analysis, mice (101/El x C_3H/El) were whole body irradiated with a single dose of 2 Gy Cs-137 gamma rays. They have been sacrified after 21 and 35 days post irradiation and samples were taken. Measurements are made with the TV-microscope using the 100x objective (oil immersion). Physical resolution is about 1 micron (MTF 0.5). In the blood smears 50 lymphocytes and 50 granulocytes were interactively selected and measured with a digital resolution of 0.25 micron pixel size. In the sperm specimens 200 spermatozoa were measured after image zooming with a digital resolution of 0.1 micron pixel size. The cells were reclassified into 3 classes: control, 2 Gy - 21d, 2 Gy-35d by multivariate discriminant analysis. Confusion matrices are shown in tables I - III:

Table I: 3-class case of sperms with shape parameters only

	Control	Day 21	Day 35	% correct
Control	724	34	46	90.0 %
Day 21	101	734	316	64.7 %
Day 35	93	249	460	57.4 %

Table II: 3-class case of sperms with chromatin features

	Control	Day 21	Day 35	% correct
Control	749	2	50	93.5 %
Day 21	81	931	194	77.2 %
Day 35	64	175	561	70.1 %

Table III: 3-class case of lymphocytes with chromatin features

	Control	Day 21	Day 35	% correct
Control	129	35	33	65.5 %
Day 21	13	126	59	66.3 %
Day 35	21	46	132	66.3 %

The results demonstrate the existence of marked morphometric and photometric changes in both sperms and white blood cells in mice after a whole body dose of 2 Gy. The sperm shape assay seems inferior to chromatin measurements.

IV. Objectives for the next reporting period:

1) Completion of 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. Hartmann, DKFZ Heidelberg
Dr. Menzel, University Homburg
Dr. Rechenmann, INSERM Straßbourg

VI. Publications:

Leuthold, G., Burger, G.: Mathematical simulation of proton tracks in water vapour and their microdosimetric analysis (submitted to Rad. and Environm. Biophysics) (1988)

Leuthold, G., Burger, G.: Dose mean lineal energy for fast neutrons in small spherical targets. Proc. 6th Symposium on Neutron Dosimetry, Neuherberg 1987 (in press) (1988)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Prof. Dr. W. Jacobi
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GSF
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Dr. H.G. Paretzke
Institut für Strahlenschutz
GSF
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:

Dr. H.G. Paretzke

Scientific staff:

Dr. K. Long, S. Heß

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 (cells, 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:

- Improvement and implementation in track structure code of photo-absorption, - coherent and -incoherent scattering cross sections for 15 elemental and molecular materials of importance in radiation biophysics (including liquid water and DNA) from 1 eV to 100 MeV,
- Calculation of electron and positron inelastic scattering cross sections and stopping power for water molecules of increasing density (from quasi-free molecules in gas to dense liquid clusters),
- Further testing of complex geometry computer routines needed to simulate heterogeneous biological targets and improvement of their speed of computation,
- Identification of useful sets of quantities describing the relative biological effectiveness of different fields of ionizing radiation

Methodology:

- Various sets of literature data on photon scattering cross sections were evaluated, checked for consistency with other integral data and the data chosen were prepared in a tabular form adequate for fast computation and minimum storage requirements.
- The local density approximation for an interacting electron gas was used to calculate charged particle scattering in an isolated molecule and in a 'molecule' within condensed matter where the other molecules are arranged according to the pair correlation function derived by molecular dynamics simulations for liquid water.
- The complex geometry computer routines were tested by comparison of computed results for the location of points deterministically migrating on straight lines through the bodies (within/without body) with analytical solutions.
- Several quantities (specific energies in small sites of various dimensions, LET_q with varing q, associated clusters, etc.) were tested for their usefulness to explain experimental cellular inactivation (mammalian cells) data for various radiation fields.

Results:

- The photon scattering cross sections for all materials, energies and types of interaction (differential in angle and target shell) are now available in a form optimized for speed of computation and memory space needed.
- In cooperation with Diercksen et al., Max-Planck-Institut für Astrophysik, Garching, the electronic properties of a single water molecule and a n = 5 cluster of water molecules has been calculated using an LCAO-approach with linear combinations of Gaussian orbitals, and from this the double differential cross section for secondary electron emission were derived. First the case of positrons was considered (where no exchange effects have to be taken into account); fig. 1 shows results obtained for the stopping power of such positrons in single water molecules and at high molecular density. The results for electrons in water vapour show differences to the experimental results of Bolorizadeh and Rudd, which most likely is due to remaining problems in our theory as well as in the experimental data.
- The complex geometry algorithms describing the simulated bodies are accurate enough for the intended purpose; in some cases the speed of computation has to be improved.
- Intercomparisons of calculated results for various sets of potentially classifying track structure quantities with experimental cell inactivation data indicate that, a), there are basic differences in the biological natures of primary relevant events produced in mammalian cells by irradiation with high and low LET-radiations, and, b), that it is necessary to consider for classification both the microstructure of clusters within cell nuclei and the total number of clusters exceeding certain sizes within the same nucleus.

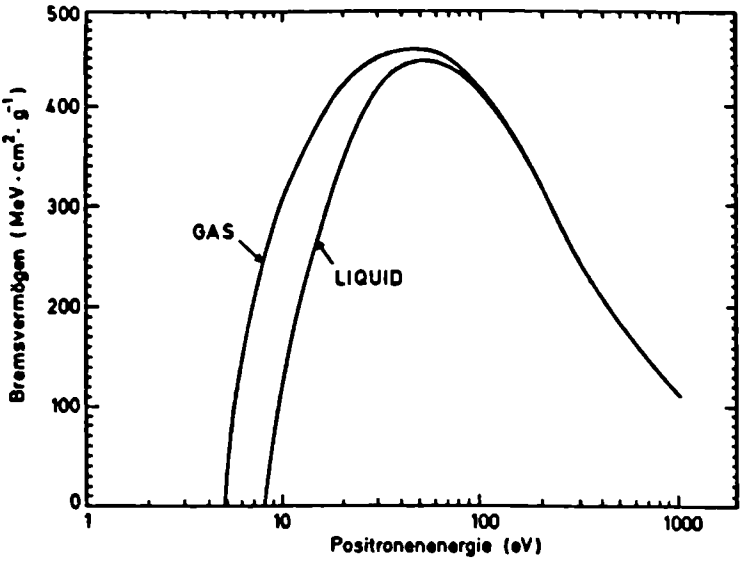


Fig. 1: Calculated stopping power for positrons in water calculated

Discussion

- The implementation of the photon interaction cross sections has been completed now.
- The theory develloped for the derivation of electron and other charged particle inelastic scattering cross sections show the right trends with increasing targed density. However, the absolute values do not yet agree well with all reliable experimental data (e.g. on binding energies measured in ESCA-work), and means to improve this agreement have to be looked for.
- The complex geometry computer routines appear to work well; more realistic bodies simulating DNA coiled around histones should now be develloped.
- Futher work is needed to improve classification schemes particularly taking energy migration along affected molecules into account.

IV. Objectives for the next 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.

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:

1) J. Booz, H.G. Paretzke, E. Pomplum, P. Olko:
Auger-Electron Cascades, charge potential and microdosimetry of iodine-125.
Radiat. Environ. Biophys. 26 (1987) 151-162.

2) W.E. Wilson and H.G. Paretzke:
An Analytical Model for Ionization Distributions Produced in Nanometer Volumes by Recoil Protons.
Accepted by Radiat. Prot. Dos. (1987).

3) W.E. Wilson, N.F. Metting, H.G. Paretzke:
Microdosimetric Aspects of 0.3 to 20 MeV Proton Tracks-I: Crosses.
Submitted to Radiat. Res.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

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

Contract no.: BI6-A-013-D

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.
2. Computational studies for the twin detector method.
3. Mathematical and numerical studies in microdosimetry.
4. 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, Dr.R.Greinert, H.Friede

I. Objectives of the project:

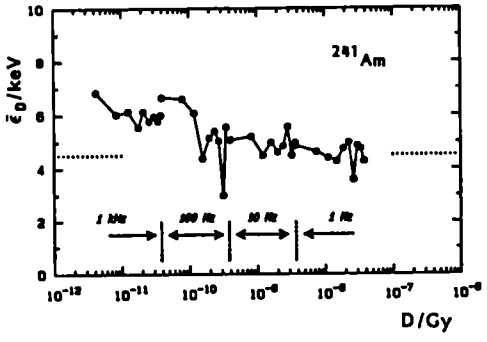
The variance-covarinace method (Kellerer and Rossi, Radiat.Res.97, 237, 1984) permits the determination of microdosimetric parameters in radiation fields of fluctuating intensity. A pair of detectors (the twin detector) is exposed to the same radiation field and registers the energy imparted within each 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.

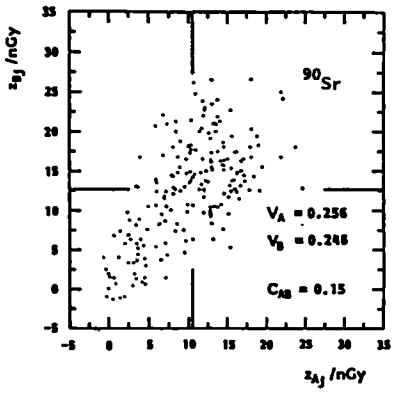
II. Objectives for the reporting period:

The prototype experimental set-up which had been developed in the first reporting period has now been employed in time-varying radiation fields for which the conventional variance method is not suitable. The measurements performed in radiation fields of low dose rate show the applicability of the method and the advantages, such as facility and speed of measurement.

III. Progress achieved:

Measurements were performed with a low dose rate Am-241 source (3.5 MBq, 4 nGy/s) and with Sr-90 (33 MBq, 300 nGy/s) and Cs-137 (185 MBq, 750 nGy/s). It has been shown that the determination of the dose mean energy imparted, $\bar{\varepsilon}_D$, the specific energy, $\bar{z}_D$, or the lineal energy, $\bar{y}_D$, can be performed over a wide range of absorbed dose and dose rate (about 4 orders of magnitude in the left figure, but can be expanded up to 6 orders of magnitude).





The values obtained in fluctuating radiation fields are consistent with results at constant dose rate, and with single-event measurements. The right figure shows, as an example, the correlation diagram of the specific energies, z, in the two detectors of the twin device for a time-varying Sr-90 field. The covariance, C_{AB}, contributes the predominant part of the variances, V_A and V_B. The derived value of $\bar{\varepsilon}_D$ agrees well with that obtained for C_{AB} close to zero (constant dose rate).

IV. Objectives for the next reporting period:

The irradiation and detector geometries in the prototype measurements do not guarantee the uniform fluence throughout the sensitive volume of the counters for which the microdosimetric variables ϵ, z, and y are usually considered . Furthermore, the present detectors are not tissue equivalent. These limitations will have to be removed to extend the work beyond the methodological developments. In the next phase of the work more suitable radiation sources will be employed and a tissue equivalent twin-detector device will be deployed. Subsequent measurements will be performed also in a pulsed accelerator beam.

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 and M.Zaider).

VI. Publications:

Breckow, J., Wenning, A., Roos, H., The Variance-Covariance Method . Experimental Realisation and Tentative Applications. IMSK 86/110.

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

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, Dr.R.Greinert, Dr.L.Hieber

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:

Past work has led to the identification of close connections between theorems involving the proximity function in microdosimetry and analogous results which are relevant to other applications. One problem of particular interest is that of systematic sampling which is important in stereology. The application of the concept of the proximity functions to such problems has, therefore, been an objective in this research period.

III. Progress achieved:

A fundamental theorem in microdosimetry links the proximity functions of the charged particle tracks and of the reference site with the energy weighted mean event sizes of y, z or y. The formula is a general relation for the variance of the intercept of two randomly superimposed objects. This generality has permitted various extensions of the theorem which has first been developed for the microdosimetric application. A new field of application has now been the well known lattice problem, more broadly, the problem of area or volume estimation by systematic sampling.

In this and related problems there has long been an interest in periodic or nearly periodic oscillations of the magnitude of the variance which result when the resolution of the test system is increased. Matheron, who has made important contribution to stochastic geometry termed this the 'Zitterbewegung'. In the work of the last research period, closed expressions have been found for these dependences, and criteria have, for the first time, been developed for the presence or absence of the oscillations. A condition for their presence is the existence of discontinuities in the chord-length distribution of the specimen.

The studies have employed Euler's method of successive approximations in terms of the Bernoulli polynominals to the fundamental relation in terms of the proximity functions. While the Bernoulli polynominals provide solutions in the case of systematic planar sectioning, a more general method is now considered which permits similar procedures for more general test systems, and especially for point sets.

IV. Objectives for the next reporting period:

A generalization of Euler's method of successive integrations by parts to a broader class of functions will be the main objective in the next research period. This involves further studies of the classical lattice-point problem.

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 and M.Zaider).

VI. Publications:

Kellerer, A.M., On the Variance of Volume Estimators from Systematic Planar Sections. Submitted to the Journal of Microscopy.

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:

Aim of the present experimental investigations is the determination of radial distributions of energy in particle tracks of higher velocity than previously investigated.

Measurements of the radial energy distribution, for protons between 4 MeV and 11 MeV, were carried out.

III. Progress achieved:

Experimental work on the determination of lateral distributions of nitrogen light-emission in lineal particle beams was continued. The calibration and testing of the instrumentation has been completed. Numerical results on the lateral distribution of light emission around proton beams have now been obtained. These new data are of interest primarily because they permit comparisons with computational results. The proton energy was varied between 4 MeV and 11 MeV. The tandem-accelerator at the Physics Department of the University of Erlangen was used. Beam intensity was monitored with a Faraday-Cup. An example of the measurements is given in Figure 1. The measured light-intensity distribution of 11 MeV-protons is shown, for seven different pressures of nitrogen from 0.53hPa to 227hPa (corrected for collisional quenching). The left curve represents the first negative system at 391.4nm, the right curve the second positive system at 337.1nm.

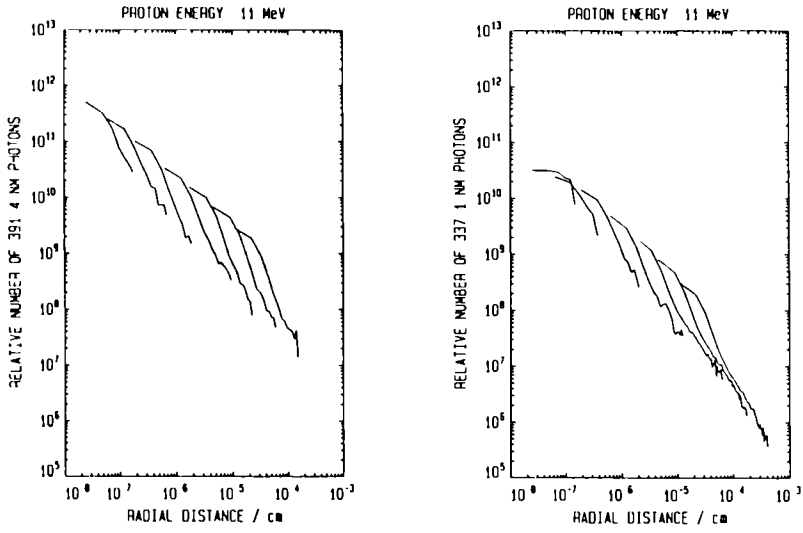


Fig. 1

For the interpretation of the experimental data their Abel-inversion is required, and this was achieved by a new optimisation method utilizing the generalized reduced gradient algorithm (GRGA). Figure 2 shows the Abel inverted specific emission density normalized to 1g/ccm. The dependence on radial distance is constructed from segments obtained at different pressures (left curve: first negative system; right curve: second positive system). At small distances the influence of the finite beam

width becomes apparent. One concludes, that it is possible to cover seven orders of magnitude in emission density and four orders of magnitude in radial distance. At present the measurements are limited to a maximum of 5µm equivalent radial distance, due to accelerator produced radiation background.

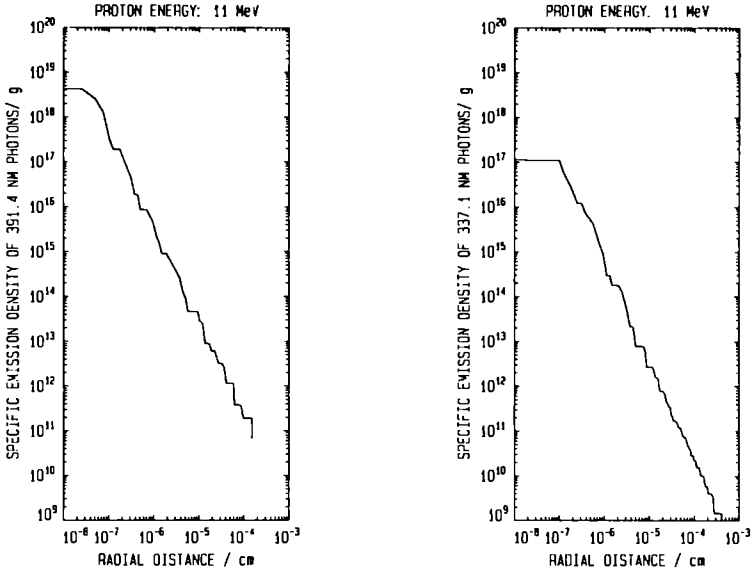


Fig.2

Multiplying the ordinate values of the first negative system by a factor of $7.8\ 10^{-14}$ one obtains the absorbed dose in gray.

The results are now being compared to corresponding data from a simulation code.

IV. Objectives for the next reporting period:

After the termination of the experimental studies for the determination of radial energy distributions, numerical comparisons will be performed with data from charged particle-track simulations.

The problem of the background noise at higher ion energies will require special attention.

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,
Physikalisches Institut der Universität Erlangen,
Gesellschaft für Schwerionenforschung (GSI), Darmstadt.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
K.H.Chadwick
H.P.Leenhouts

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:

- Calculation of DNA damage induced by different radiation types, using the modified track model;
- Application of the model to calculate the effect of monoenergetic neutrons on eukaryotic cells;
- Analysis of experimental data in terms of the molecular hypothesis of radiation biology;
- Confirmation of preliminary results on the fractionation and delayed plating effects after irradiation of stationary CHO cells with UV and comparison with results using X-rays.

III. Progress achieved:

Track structure model:

The modified track structure model has been applied to calculate the efficiency of different types of radiation for the production of DNA strand breakage, i.e. of both single strand breaks and double strand breaks induced by the passage of one ionizing particle. The track structure model gives information on energy deposition events along the path of an ionizing particle at nanometer dimensions and the calculations take into account the physical dimensions of the double helix structure of the DNA molecule. The data of Ritter et al. (Nature, 266, 653-655 (1977)) have been reanalysed and calculations of the relative track efficiency for double strand break induction as a function of electron energy have been made. Results show that electrons with an energy between 50 and 500 eV are the most efficient.

The track structure model has also been applied to data on cell survival after mono-energetic neutron irradiation (data: Hall et al. Rad.Res. 64 245-255 (1975) and Kellerer et al. Rad.Res. 65 172-186 (1976)) assuming that the initial slope of the cell survival curve ($p\alpha$ D-term) is related to the production of DNA double strand breaks. The model, which only takes recoil protons into account as yet, gives a good fit to the experimental data (see fig. 1) showing that neutrons with an energy of about 400 keV are most effective. The fitting parameters indicate that the damage is caused by water radicals induced very close to the DNA (0.5 nm) or by direct action of radiation on the DNA molecule.

Interpretation of non-stochastic effects using the molecular hypothesis for cellular radiation biology.

The molecular hypothesis for radiation biology has been developed in previous contracts to explain cellular effects of radiation such as cell killing, chromosomal aberrations and mutations assuming that DNA double strand breaks are the crucial lesions. Some non-stochastic effects appear to be the consequence of an accumulation of cellular effects, e.g. animal lethality: The cellular model has therefore been extended to take into consideration the non-stochastic effects and mathematical formulas have been derived as a basis for the analysis of animal data, using the α- and β-coefficient of cellular effects. At the cellular level, dose rate changes affect the β term whereas radiation quality affects the α term. Analysis of animal survival has indicated that these changes with dose rate and radiation quality are reflected at the animal level. This

extension of the model provides an association between molecular damage and repair, cellular biological effects and effects at the whole animal level.

Cellular response to X-rays and UV

The biological models derived to explain the effects of X-rays and UV both give similar cell survival equations but are based on somewhat differing mechanisms. X-ray survival is linear-quadratic with dose, UV survival is predicted to be purely quadratic with exposure. Theoretically the models predict a different behaviour of survival, when immediate, delayed and fractionated irradiations are compared. The experimental protocol was acute irradiation with immediate plating (i) and with 24 hr delayed plating (d) and fractionated irradiation (f) where the second fraction was given after 24 hr immediately followed by plating. The theoretical predictions and experimental results using stationary CHO cells are shown in fig. 2. The different predictions are dependent on differences in the behaviour of the quadratic dose terms and the results are interpreted to be in support of a genuine 'two-hit' component in X-ray survival curves.

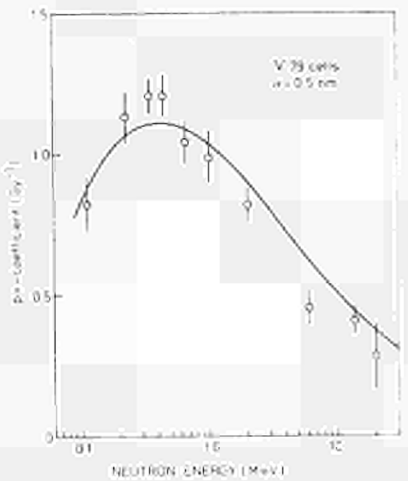


FIG.1. The dependence of experimental α-coefficients of V 79 on neutron energy and the fit of model using an interaction distance σ = 0.5 nm.

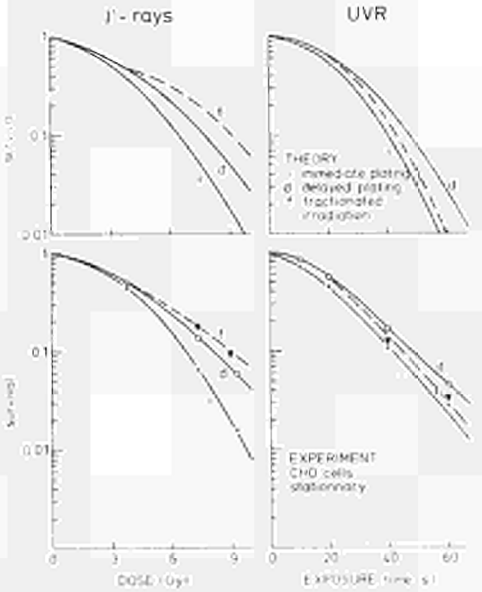


FIG.2. Theoretical predictions and experimental results on X-ray and UV-irradiated CHO cells (see text for experimental protocol).

IV. Objectives for the next reporting period:

- Analysis of cell survival and transformation data after exposure to high energy ions (100-1000 MeV/u, 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.

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

- Drs.J.M.Nelson, L.A.Braby and N.F.Metting, Pacific Northwest Laboratory Richland, USA.
- Drs.A.Cebulska-Wasilewska and M.Waligorski, Institute for Nuclear Physics, Krakow, Poland.

VI. Publications:

Leenhouts, H.P. and K.H.Chadwick

Fundamental aspects of the dose effect relationship for ultraviolet radiation. In: "Human exposure to ultraviolet radiation: Risks and regulations" (eds. W.F.Passchier, B.F.M.Bosnjakovic) Elsevier, Amsterdam (1987) 21-25.

Chadwick, K.H. and H.P.Leenhouts

DNA damage and chromosome aberrations. In: "Radiation Carcinogenesis and DNA Alterations" (eds. F.J.Burns, A.C.Upton, G.Silini) Plenum Publ. Corp. N.Y. (1986) 245-264.

Chadwick, K.H. and H.P.Leenhouts

Chromosome break-points, somatic mutation and oncogene activation: some comments. In: "Radiation Carcinogenesis and DNA Alterations" (eds. F.J.Burns, A.C.Upton, G.Silini) Plenum Publ. Corp. N.Y. (1986) 265-276

Leenhouts, H.P. and K.H.Chadwick

The yield of chromosomal aberrations and its correlation with other biological endpoints. In: "Radiation Carcinogenesis and DNA Alterations" (eds. F.J.Burns, A.C.Upton, G.Silini) Plenum Publ. Corp. N.Y. (1986) 277-291.

Leenhouts, H.P. and K.H.Chadwick

Track structure and single hit detectors. In: "Radiation Research". Proc. 8th Int.Congress Radiation Research. Taylor and Francis, London. vol. 1 p. 84 (Abstract).

Leenhouts, H.P. and K.H.Chadwick

On the common nature of the cytotoxic lesion. In: "Radiation Research". Proc. 8th Int. Congress Radiation Research. Taylor and Francis, London, vol. 1 p. 63 (Abstract).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 - Laboratorio 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. Neutron dosimetry by damage track detetor: Advanteges and limitations.

Head(s) of project:

L.Tommasino

Scientific staff:

L.Tommasino, G.Torri

I. Objectives of the project:

The solutions of many problems in neutron dosimetry (respectively for personnel, area and environmental monitoring) may be achieved through the optimization of the electrochemical etching parameters such as Electric Fields, Temperature and Frequency. The major scope of this project is to demonstrate how it possible to obtain any desired neutron-dosemeter response through a proper choice of these parameters.

II. Objectives for the reporting period:

Investigation of the electrochemical etching parameters for the optimization of CR-39 detector response to neutrons will be continued. Particular efforts will be made to study the neutron response of CR-39 with large backgrounds after two-steps etching.

III. Progress achieved:

The most important characteristic of the electrochemically etching process is the ability to control the etching procedures with an external apparatus to obtain any desired neutron detectors response.

In 1987 the choise of etching parameters has been made in order to reduce the detectors background and to obtain any desired neutron detector response with limited track overlapping and small dependence on neutron energy.

The optimization of these parameters has proved to be successful using (among other things) samples irradiated in the Joint European/USA/Canadian irradiation organized by EURADOS-CENDOS.

In particular the etching parameters used are 3 hours pre-chemical etching at 25°C. Under these etching conditions, the sizes of the track spots are relatively uniform when compared with those obtained etching only electrochemically at high temperatura (60°C). It was thus possible to count only spots with sizes greater than a certain value thus eliminating part of the background spots.

In this way it was possible to improve the signal-to-noise ratio for materials charaterized by relatively large background.

IV. Objectives for the next reporting period:

Since the background of CR-39 detectors till represents the most important start coming for neutron dosimetry, investigations on the optimization of the etching paramters will be continued with the major mission goal of reducing this background.

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

R.V.Griffith - Lawrence Livermore Laboratory - USA
W.G.Cross - Chalk River Nuclear Laboratory - CANADA

VI. Publications:

L.Tommasino - Future developments in etched track detectors for neutron dosimetry - Rad. Prot. Dos. 20, 121-124 (1987).
D.Azimi Garakani, B.Flores, L.Tommasino and G.Torri - Intercomparison results of the Cendos Irradiations. EURADOS-CENDOS Report 1987 - 01.

Title of the project no.:

Project N.2 - Applied Personal Dosimetry by Chemically Etched CR-39 Dosimeters.

Head(s) of project:

Dr. L. Lembo

Scientific staff:

L. Lembo, O.Civolani, M.Beozzo

I. Objectives of the project:

Development of a CR-39 Neutron Personnel Dosimeter for Large Dosimetry Services.

II. Objectives for the reporting period:

Construction and setting up of the various devices required to transfer the track etch detectors using the electrochemical technique in routine neutron dosimetry.

Study of dosimetric characteristics of chemically and electrochemically etched CR-39 detectors.

III. Progress achieved:

During 1987 the activities developed have been mainly devoted to the construction and setting up ot the various devices required to trasnfer the track etch detectors using the electrochemical technique in routine neutron dosimetry and to investigating some dosimetric characteristics of chemically and electrochemically eteched CR-39 detectors.

In particular 10 lucite cells, each capable of simultaneously eletectrochemical etching 24 CR-39 detectors, 30x 28 mm^2 in dimension and 600 μm thick, have been designed and made. During the etching cycle, usually beginning early in the morning, 4 of these cells are placed inside an oven maintained at a temperature of 60°C, where they are automatically filled with the KOH solution by means of a hydraulic circuit. When the etching cycle, controlled by a HP pocket computer, is ended the cells are automatically emptyed and cleaned several times with running water. After the cleaning cycle, the cells are allowed to dry in the oven overnight. In this way the whole etching cycle is automatically performed, with no need for the operator to manipulate the etching solutions or control the etching conditions.

The detectors are one-side etched, while, on the opposite side a bar code identification label is affixed. Track evaluation is performed with a small, cheap image analyser; this instrument and a bar code reader are both connected to a PC computer for data recording and dose calculation.

We plan to introduce this new technique in our personnel dosimetric service early in 1988, substituting the currently used technique based on nucleare emulsion.

As for the study of CR-39 dosimetric characteristics, a systematic investigation of the background with respect to storage time and batch purchasing, has been performed.

CR-39 materials from American Acrylics Plastics, 600 μm thick, masked with polyethylene film on both sides, have been investigated. The experimental results showed that the background of samples bought in January 1987 and stored in a refrigerator at about 0°C, increased from about 60 cm^{-2} at about 800 cm^{-2} after nine months, and the sensitivity at Cf-252 neutrons decreased by about 20% in the same period. A new CR-39 batch purchased from the same manufacturer in July, 1987 has shown a background equal to 200 cm^{-2}, much higher than the corresponding one of the first batch. All the experiments have been performed in a 6.25 N KOH solution at 60°C, using the following etching conditions: 6 hours at

35 $kVcm^{-1}$ 50 Hz plus 2 minutes 35 $kVcm^{-1}$ 2000 Hz, followed by 15 minutes of normal chemical etching.

Using the above etching conditions, we participated in the neutron irradiation intercomparison experiment organized in 1986-87 by Eurados-Cendos. Fig. 1 shows the energy dependence we obtained with this experiment using CR-39 detectors with no additional radiator and etched only on the back.

After a visit to NRPB laboratories in November 1987, comparison of our background measurements with those performed by NRPB, emphasized the need to investigate the possibility of reducing the value of the electric field used during our etching cycles, with the aim of obtaining a lower and more reproducible background without losing too much in sensitivity and energy response.

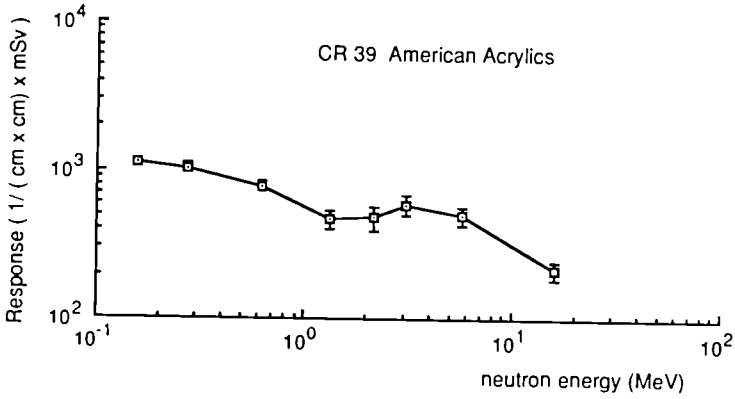


Fig. 1

IV. Objectives for the next reporting period:

Introduction of the developed CR-39 dosimetric system for routine personnel dosimetry. Investigations for obtaining a lower and more reproducible background of CR-39 detectors by reducing the electric field used during the etching cycles.

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

D.Bartlett, NRPB, HARWELL (U.K.)

VI. Publications:

E.Piesch "Neutron irradiation of proton-sensitive track detectors: results of the joint European/USA/Canadian irradiation organized by Eurados-Cendos (1986)" - KfK 4305 EURADOS-CENDOS REPORT 1987-01, Kernforschungszentrum Karlsruhe GmbH, Karlsruhe.

Title of the project no.:

3. The assessment of low concentration of alpha emitting radionuclides.

Head(s) of project:

G.Sciocchetti, L.Tommasino, G.Torri

Scientific staff:

F.Breuer, G.Cotellessa, G.Sciocchetti, L.Tommasino, G.Torri

I. Objectives of the project:

Development and applications of new detectors based on track-structure properties (damage track detectors and silicon-based devices) for the assessment of low concentrations of natural and man-made alpha emitting radionuclides in man and IN environment.

II. Objectives for the reporting period:

A. Man-made alpha emitters
Simultaneous measurements of alpha particles on neutron-induced fission for the identification and characterization of plutonium bearing particulate from Chernobyl.

B. Natural alpha emitters
Continuation of radon measurements both in indoor and outdoor environments and automation of the reading system. Development of a microprocessor-based radom sampler and dosemeter.

III. Progress achieved:

A. Man-made alpha emitters

Membranes used for the filtration of large-amounts of air from the Chernobyl accident have been gathered both from Italy and Sweden. These filters are now been exposed for the assessment of low concentrations of alpha emitters.

The next step is to irradiate the same filters to large thermal neutron fluxes to measure the neutron-induced fission fragments.

The simultaneous measurements of alpha particles and neutron-induced fission will be used for the characterization of plutonium bearing particles from Chernobyl.

B. Natural alpha emitters

The scopes of large scale surveys of indoor radon may be very different. There are "screening" surveys which are required to investigate the geographical variations in the country or region, while other surveys are designed to obtain the average population doses for use in epidemiological studies.

In this second case, measurements of long-term radon concentration are needed which can be conveniently obtained with all available monitor devices based on damaged track detectors.

For screening applications, an exposure period as low as one week is required. For these measurements, damage track detectors are considered not sufficiently sensitive and the diffusion barrier charcoal absorption colletor, DBCA, is used in spite ot its reponse highly dependent on temperature and humidity.

In 1987 project it has been possible to demonstrate how simple is to measure short-term radon exposure by spark counting large detector areas. In particular, by using 100 cm^2 cellulose nitrate foil, the detection limit becomes as low as 8 Bq/m^3 for one week exposure. Using the long-term radon monitor devices, a large scale surveys of soil radon has been made throughout Latium and Campania.

For several locations, results of measurements gathered for different years have been analysed.

Appropriate software for alpha track automatic reading of radon-dosimeters has been developed.

IV Objectives for the next reporting period:

Man-made alpha emitters

Characterization of plutonium bearing particles from the Chernobyl accident.

Natural alpha emitters

Indoor radon survey both for monthly and weekly variations of radon concentrations in typical houses.

Setting up of a complete system for the electrochemical etching and automatic reading to process a large number of passive detectors for extensive survey of radon air concentration.

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

1. D.E.Cherouati and M.Dahmana, Commissariat aux Energie Nouvelles, Algeria.
2. J.L. Seidel and M. Monnin, CNRS, Clermont-Ferrand, France.
3. B.Flores, University of Riobamba, Ecuador.
4. D.Azimi-Garakani, University of Tehran, Iran.

VI. Publications:

D.Azimi-Garakani, B.Flores, S.Piermattei, A.F.Susanna, J.L.Seidel, L.Tommasino and G. Tossi - Radon-gas sampler for indoor and soil measurements and its applications, Paper presented at the IV International Conference on Natural Radiation Environment, Lisboa, 7-11 Dec.1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

1. Investigation into the choice of phantom shape and material for the calibration of personal dosemeters in terms of the new operational quantities.

Head(s) of project:
Dr D T Bartlett
Instrumentation Department
NRPB
Chilton, Didcot
Oxon, OX11 ORQ UK

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 operational quantities for external radiation exposure require the choice of suitable phantoms on which to calibrate personal dosemeters when determining their energy and angular response. ICRU have indicated that the ICRU tissue equivalent sphere is a suitable phantom for dosemeters designed to be worn on the body. Material of ICRU soft tissue composition cannot be produced. Also the 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 investigations, both by calculation and experiment, of the dependence of the response of personal dosemeters on the shape and material of phantoms used, or to be used, for calibration or type testing.

III. Progress achieved:

1. Methodology

Calculations have been performed of the dose equivalent distributions inside and the energy and angle spectral distributions of the backscattered radiation field at the surface of phantoms of interest for incident photon radiation. The calculations employ a Monte-Carlo code 'DEIPHOS' developed at NRPB. In general, sufficient histories are accumulated to reduce the statistical uncertainty to less than 5%. The program is run on a VAX 785 at NRPB Chilton.

The purpose of these calculations of backscatter radiation fields is to provide data to be able to predict the response of personal dosemeters irradiated on the phantoms with sufficient accuracy to assess the suitability of the phantoms as substitutes for an ICRU sphere for calibration and type testing and, where required, provide correction factors. Calculations have also been made of the backscattered radiation field at a distance of 1 cm from the phantom surface. The results of these calculations provide an estimation of the dependence of the response of a dosemeter on its separation from the body.

The purpose of the calculations of dose equivalent at a depth of 10 mm in phantoms of ICRU 4-element tissue other than the 30 cm sphere is to assess the importance of the effect of phantom geometry on the ratio of the response of a personal dosemeter to the dose equivalent at 10 mm. Examination of the effect of phantom geometry provides a means of assessing the suitability of the use of the ICRU sphere for the calibration of personal dosemeters whose aim is to measure Hp(10) when worn on a body of markedly different shape to the ICRU sphere.

2. Results

The calculation of the response as a function of incident photon energy and angle of a minimal design of personal dosemeter and its comparison with H'(10) have been completed, analysed and published. The results show the importance of the lateral extent of

the covering material over the detector for normal incidence at some energies as well as more generally for other angles of incidence.

Calculation of backscattered fluence and planar fluence energy and angle spectral distributions have been completed for monoenergetic photons (broad beam) incident at angles (radians) of $\cos^{-1}$ 0.1 increments on 20, 30 and 50 cm diameter spheres of ICRU 4-element tissue and a 30 cm diameter sphere of MS20, and normally incident on a 30 cm cube of MS20, and a 30 cm cube of PMMA (Perspex or Lucite). The dose equivalent at a depth of 10 mm as a function of incident photon energy and angle has been calculated for 20 and 50 cm diameter spheres of ICRU tissue and previous results for a 30 cm diameter sphere augmented. A comparison has been made between the results obtained of backscattered radiation energy and angle spectral distribution for a 30 cm sphere of ICRU 4-element tissue with results of similar calculations for the same phantom obtained by PTB. There was good agreement. Accordingly, calculations have not been performed for water or polyethylene phantoms : it is proposed to use the results of such calculations performed by PTB. A 30 cm cube of PMMA is being constructed.

Measurements have been made with thermoluminescent detectors mounted on the front surface of the MS20 cube phantom, to validate the calculations.

IV. Objectives for the next reporting period:

During the next reporting period a full analysis of the results will be made and a report prepared.

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

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).

VI. 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).

Title of the project no.:

2. Field measurements of photon energy and angle spectral distribution, the determination of ambient dose equivalent penetrating, and effective dose equivalent, and their comparison.

Head(s) of project:
Dr D T Bartlett
Instrumentation Department
NRPB
Chilton, Didcot
Oxon, OX11 0RQ, UK

Scientific staff:

Dr D T Bartlett
Mr P H Burgess
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 individual dose equivalent penetrating.

II. Objectives for the reporting period:

The objectives for the reporting period January to December 1987 were to analyse and report the results of the complete set of measurements.

III. Progress achieved:

1. Methodology

The photon spectrometer constructed for and used in these studies consisted of four Geiger-Muller tubes provided with various filters which result in distinctly different energy responses, fitted into lead collimators to define an acceptance angle for lower energy photons. Photon energy spectra could be crudely analysed in terms of energy 'bins': 10 to 40 keV, 40 to 100 keV, 100 to 180 keV, 180 to 600 keV and 600 keV upwards. High energy components where the energy exceeded 2 MeV could be identified. For photon energies up to 180 keV, the angular distribution of the field was measured in conical 'bins' of cone half angle $\pi/4$. The energy and angle dependence of response of the spectrometer was characterised using the ISO Narrow and Fluorescent series of radiation fields and the deconvolution program tested using simulated responses to theoretical spectra.

When making field measurements, the direction of the highest intensity (in terms of air kerma) was identified and six measurements made in the orthogonal direction defined by this direction and the horizontal and vertical planes. The time for a measurement was such as to accumulate at least 1000 counts in each of the detector. Backgrounds are typically 0.3 counts s^{-1} and therefore air kerma rates of greater than 1 $\mu Gy\ h^{-1}$ were required in order to obtain a satisfactory signal to noise ratio.

The measured count rates are deconvoluted to give a crude energy and angle spectral distributions of air kerma rate. These distributions will be folded with conversion coefficients to ambient dose equivalent at 10 mm, directional dose equivalent at 10 mm, and to instrument and dosemeter responses.

2. Results

Field measurements have been made at 6 sites in each of 3 buildings at BNF plc Sellafield, 8 sites at Amersham International, a simulated fluoroscopy examination and at an industrial radiography facility. With the exception of the fluoroscopy examination, no

significant low energy components (E < 100 keV)) were identified. However, in all cases where fissioning material or other neutron emitting sources were present in significant quantities, a high energy component (E > 2 MeV) was noted. The analysis of the results is in hand and a full report in preparation.

IV Objectives for the next reporting period:

The objectives are to prepare a full report, and to publish the results with a discussion of the implications for choice of monitoring quantities, instruments and dosemeters.

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

None.

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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. Thermoluminescence dosimetry studies.

List of projects:

1. Calculation of doses from external radiation.
2. Investigation of methods for improving the thermoluminescence properties of thin dosemeters for application to beta and low energy photon dosimetry.
3. A study into the development of multiple dose re-assessment in thermoluminescent materials for high dose measurement and into factors affecting the efficiency of both optical and thermal transfer and recombination processes in these materials.

Title of the project no.: 1

Calculation of doses from external radiation

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:

(1) Improvements in the modelling of phantom and dosemeter. In particular the introduction of an overlying absorber on the dosemeter so that it more accurately simulates a real dosemeter.

(2) Dose comparisons in other field configurations.

(3) Further work on scattered radiation fields.

III. Progress achieved:

Work on the comparison of personal dosemeter readings with organ doses and effective dose equivalent for parallel beam irradiation was considerably extended. Calculations were carried out for AP, PA and lateral directions and for a complete rotation about the phantom in a horizontal plane. The range of energies used was from 10 keV to 10 MeV and the phantom was based on the Cristy (1980) adult hermaphrodite model with some small modifications.

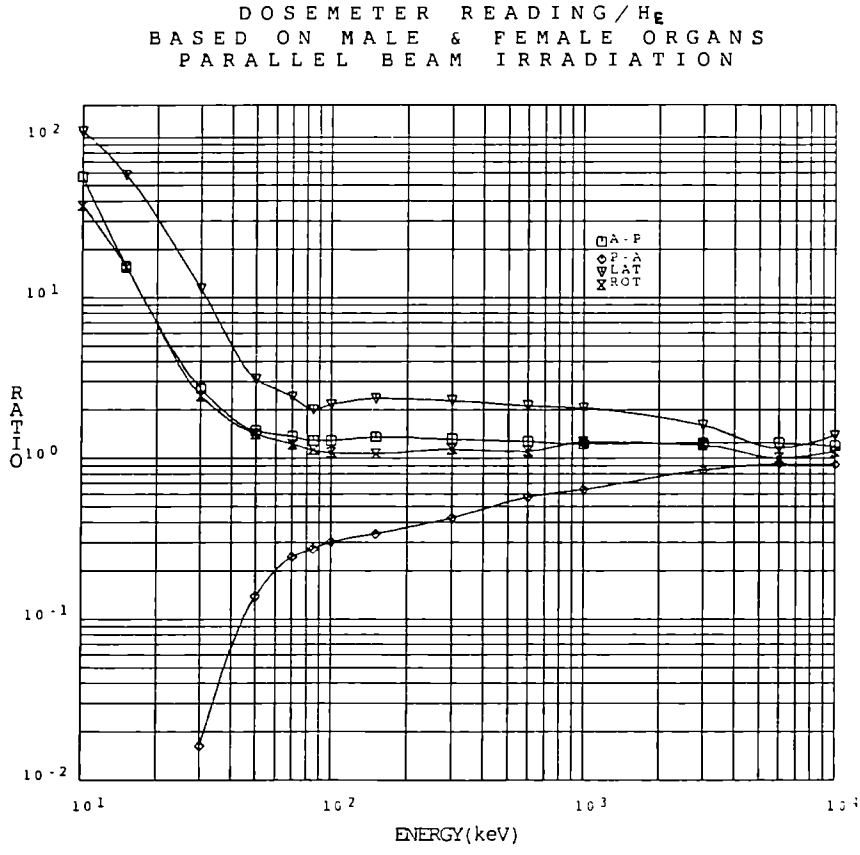
The effective dose equivalent (H_E) was calculated and compared with the dose recorded by an idealised dosemeter placed on the phantom. This dosemeter was located on the midline of the anterior of the body 14 cm above the bottom of the trunk. Some of the calculations were repeated with a 1 cm thick tissue equivalent absorber placed on top of the dosemeter, but this had little effect for radiation of energies above a few tens of keV. The relationship between H_E and dosemeter reading is shown in the figure.

The relationship between H_E and kerma in air and between some selected organ doses and kerma in air were determined and compared with some results calculated by Williams et al (1985) at GSF which were based on separate male and female phantoms. Expressing the differences as a percentage of the maximum value within the energy range, the results for doses to the skin, skeleton and lungs showed good agreement with maximum differences of 5, 4 and 6 percent respectively. The differences for bone marrow, gonads, breasts and thyroid were 12, 15, 18 and 20 percent respectively and may be due to the differences in the size between the GSF male and female phantoms. The breast volume in the GSF phantom is also about 50 percent larger than in the Cristy hermaphrodite. The results for the smaller organs like the gonads and the thyroid are affected by the necessarily poorer statistical precision.

References

Mathematical phantoms representing children of various ages for use in estimates of internal dose, Cristy M, Oak Ridge National Laboratory, NUREG CR-1159, ORNL/NUREG/TM-367 (1980).

The calculation of dose from external photon exposures using reference human phantoms and Monte Carlo methods, Williams G, Zankl M, Eckerl H, Drexler G, Part II: Organ doses from occupational exposures. GSF-Bericht S-1079 (1985).



IV Objectives for the next reporting period:

(1) Dose comparisons in other field configurations.

(2) The calculation of radiation fields associated with spatially distributed sources.

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

Gesellschaft für Strahlen und Umweltforschung, Munich, W. Germany.

VI. Publications:

None.

Title of the project no.: 2

Investigation of methods for improving the thermoluminescence properties of thin dosemeters for 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 Mr D J Richards (part)

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 determine the response of carbon-loaded LiF/PTFE and the Vinten extremity dosemeter to various beta sources and low-energy photons.

ii) To determine how effectively carbon-loaded LiF/PTFE discs and the Vinten extremity dosemeter evaluate directional dose equivalent (up to 60°) from various beta sources and low-energy photons.

III. Progress achieved:

LiF/PTFE discs (loaded with 5% carbon at manufacture) and Vinten extremity dosemeters were irradiated along with control TLD-100 chips (to relate the measurements to earlier studies). Three beta sources were used: $^{90}Sr/^{90}Y$; ^{204}TL; and ^{147}Pm (E_{max} = 2.27, 0.763 and 0.225 MeV respectively). Measurements were initially carried out with the TLDs mounted on a MS 20 phantom and under an aluminised mylar window (0.0085 mm thick). The measurements at normal incidence were carried out in an automatic exposure jig. The angular measurements used a jig specifically constructed for this purpose. A cross-comparison was carried out to determine any difference in scatter contributions from the sources in the two jigs. Throughout the study the same orientation of the dosemeters (with their respective controls) on the MS 20 phantom with respect to the radiation field was maintained. To ensure precision in the measurements a technique was developed for re-using the Vinten extremity dosemeter under research conditions. These dosemeters have been re-used more than 20 times with no significant degradation in response and no appreciable increase in background.

Two ISO narrow x-ray spectra with mean energies 248 and 33 keV were used for the low energy photon study. The TLDs were mounted on a MS 20 cube phantom of 30 cm sides under an aluminised mylar window (0.0085 mm thick). The cube was constructed of slabs 1 cm thick and the TLDs were mounted on the front slab. This construction ensured that the TLDs could be positioned in exactly the same location for each irradiation. Recesses were machined in the block to ensure that the TLD-100 chips (0.9 mm thick) were coplanar with the front face of the phantom. Six TLDs (either carbon loaded discs or Vinten extremity dosemeters) were irradiated on the phantom at a time along with six TLD-100 chips. The TLDs were irradiated on the axis of rotation for the angular measurements to ensure that all were exposed to the same field conditions. The quantities of interest for each radiation condition are the sensitivity of the dosemeter and its detection threshold. The sensitivity is defined as the net response in terms of counts (recorded counts minus background) per unit directional dose equivalent. The threshold is calculated at the 95% confidence level: twice the standard deviation on the mean background signal divided by the sensitivity. The results are summarised in the tables.

In conclusion, this preliminary study has shown that the energy responses of carbon-loaded LiF/PTFE discs are within 40% of unity (response to $^{90}Sr/^{90}Y$ taken as unity) for low energy beta radiation. The angular response for the three detectors studied were within 50% of the values for directional dose equivalent obtained with an extrapolation chamber. The detection threshold is ~ 2 mSv for ^{147}Pm. This high detection threshold is principally due to the low sensitivity of the detector since the variation in signal from undosed samples is comparable with that for the TLD-100 chips. Improvements in sensitivity of up to a factor of 40 may be achieved by incorporating lithium fluoride doped with magnesium, copper and phosphorous. The dose threshold would be improved by a factor of 40 if no increase in background signal resulted.

Table 1
Response of LiF TLDs to beta radiation as a function of angle
(Normalised TL counts/unit Directional Dose Equivalent)

		$^{90}Sr/^{90}Y$	^{204}Tl	^{147}Pm
TLD-100 chips	0°	1.00 (3%)	0.38 (2%)	0.03 (4%)
	30°	0.98 (2%)	0.38 (9%)	0.04 (29%)
	60°	0.73 (5%)	0.25 (13%)	0.03 (43%)
Vinten extremity	0°	0.10 (10%)	0.10 (26%)	0.02 (10%)
	30°	0.10 (4%)	0.09 (6%)	0.02 (8%)
	60°	0.09 (5%)	0.07 (3%)	0.01 (22%)
C-loaded discs	0°	0.013 (10%)	0.012 (11%)	0.008 (16%)
	30°	0.012 (15%)	0.012 (2%)	0.007 (10%)
	60°	0.011 (2%)	0.008 (21%)	0.005 (29%)

(Figures in brackets represent one standard deviation)

Table 2
Low energy photon response of LiF TLDs as a function of angle
(Normalised TL counts/unit Directional Dose Equivalent)

		248 keV	33 keV
TLD-100 chips	0°	1.00 (8%)	1.4 (13%)
	30°	1.06 (4%)	1.4 (4%)
	60°	0.94 (7%)	1.2 (3%)
Vinten extremity	0°	0.07 (2%)	0.08 (10%)
	30°	0.08 (16%)	0.09 (2%)
	60°	0.07 (10%)	0.09 (2%)
C-loaded disc	0°	0.013 (2%)	0.018 (4%)
	30°	0.013 (4%)	0.017 (2%)
	60°	0.014 (2%)	0.016 (2%)

(Figures in brackets represent one standard deviation)

Table 3
Dose threshold (μSv) (at 95% confidence level)
as a function of Beta radiation calibration source

	$^{90}Sr/^{90}Y$	^{204}Tl	^{147}Pm
TLD-100 chips	20	70	750
Vinten extremity	110	120	750
C-loaded disc	1200	1200	2000

IV Objectives for the next reporting period:
Project completed

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

VI. Publications:

Francis T M, Driscoll C M H 'Thermoluminescence beta dosimetry', NRPB Radiological Protection Bulletin, No 65, 26-29, 1985.

Francis T M 'The development of a reference instrument for the direct determination of dose equivalent from beta radiation at various depths in tissue' Radiation Protection Dosimetry, 12, 219-222, 1985.

Francis T M, O'Hagan J B, Richards D J, Driscoll C M H 'Responses of thermoluminescent materials to beta radiation and low energy photons' Radiation Protection Dosimetry, 17, 89-92, 1986.

Driscoll C M H, McWhan A F, O'Hagan J B, Dodson J, Mundy S J, Todd C D 'The characteristics of new LiF preparations and sensitised LiF' Radiation Protection Dosimetry, 17, 367-371, 1986.

Driscoll C M H, Barthe J R, Oberhofer M, Busuoli G, Hickman C 'Annealing procedures for commonly used radiothermoluminescent materials' Radiation Protection Dosimetry, 14, 17-32, 1986.

Driscoll C M H, Richards D J 'Reader annealing of LiF chips' Radiation Protection Dosimetry, 18, 99-100, 1987.

O'Hagan J B, Francis T M, Williams S M 'The re-usability of the Vinten extremity dosemeter' to be published in Radiation Protection Dosimetry 1987.

Bartlett D T, Dutt J C, Francis T M 'A new detector for the assessment of individual dose equivalent, superficial (skin dose): preliminary results' in the Proceedings of the 5th Information Seminar on the Radiation Protection Dosemeter Intercomparison Programme - Beta Dosimetry, at ENEA, Bologna, Italy, 25-27 May 1987, to be published in the EUR series, Commission of the European Communities, 1987.

Driscoll, C M H, Francis, T M, Williams S M, O'Hagan J B, Bartlett D T 'Response characteristics of carbon-loaded dosemeters to beta radiation', to be submitted to Radiation Protection Dosimetry.

Title of the project no.: 3

A study into the development of multiple dose re-assessment in thermoluminescent materials for high dose measurement and into factors affecting the efficiency of both optical and thermal transfer and recombination processes in these materials.

Head(s) of project:

Dr C M H Driscoll

Scientific staff:

Mr J B O'Hagan Mr D J Richards (part)

I. Objectives of the project:

i) To investigate phototransfer processes in a range of thermoluminescent materials including lithium fluoride, calcium fluoride and calcium sulphate in order to gain further information about electron transfer processes in these materials.

ii) To investigate methods for increasing the efficiency of the phototransfer process so that lower detection thresholds for re-assessment of absorbed dose can be achieved, and

iii) To develop multiple dose re-assessment procedures in these materials so that high primary absorbed doses can be assessed in the normal response range of luminescence detecting systems.

II. Objectives for the reporting period:

i) To determine the degree of multiple dose re-assessment from lithium fluoride (in chips and PTFE disc form), calcium sulphate, calcium fluoride and beryllium oxide.

ii) To develop a procedure for evaluating the dose received by a dosemeter which produces a thermoluminescence signal above the normal response range of the luminescence detecting systems during the primary readout cycle.

III. Progress achieved:

The multiple dose re-assessment properties of LiF (TLD 100) chips, LiF (TLD 700)/PTFE discs, CaF_2 (TLD 200) chips, $CaSO_4$ (TLD 900) chips and BeO (Thermolux 995) ceramic pellets have been studied; (the last two materials being different preparations from those reported previously[1]). The TL signals from these materials were recorded over the primary absorbed dose range 100 mGy to 1 kGy and during five cycles of re-assessment, along with the signals from undosed controls of the same type. The re-assessment parameters for the LiF/PTFE discs were a pre-heat at 115°C for 120 s, followed by exposure to ultra-violet radiation (254 nm) at 115°C for 300 s, followed by a post-irradiation anneal at 115°C for 120 s. Other materials were exposed to ultra-violet radiation under the same conditions but at room temperature.

The signals from all samples exposed to high radiation doses were above the detection range of the luminescence detecting system. The number of re-assessment cycles required to bring these signals within the dynamic range was determined as a function of absorbed dose. For all the materials, five re-assessment cycles were required to achieve this. BeO was the only material to produce negligible intrinsic photo-thermoluminescence (PTL) (<10 μGy equivalent). For other materials a computerised glow curve acquisition and analysis system was used for the subtraction of the PTL signal from the recorded signal to obtain the photo-transferred TL (PTTL) and to determine the glow curve structure due to these processes, as summarised in table 1.

The magnitude of the re-assessment signal as a percentage of the primary signal is given for each material in table 2. The re-assessment efficiency of the LiF chips is different from those of the discs due to differences in the annealing treatments applied during ultra-violet exposure.

The detection threshold D_o of each material as a function of the re-assessment cycle is given in table 3. The increase in D_o with re-assessment cycle is due to a decrease in sensitivity coupled with an increase in the variation of the PTL signal. This explains the less pronounced increase in D_o with re-assessment cycle for BeO where the PTL contribution is very low.

In conclusion, multiple dose re-assessment has been shown to be a useful technique for determining a high dose from a wide range of thermoluminescent materials when the primary signal is above the normal response range of the luminescence detecting system. It can also be used when information about the primary signal is lost, for example due to an electronic fault in the read-out apparatus. The use of a glow curve analysis system allows any PTL signal to be subtracted from the recorded signal of re-assessed dosemeters.

References

1. O'Hagan J B, Pearson A J, Driscoll C M H 'Multiple dose re-assessment in thermoluminescent materials' Radiat. Prot. Dos., 17, 347-350 (1987).

Table 1
Glow curve analysis results of the temperatures (°C) of the main glow peaks in the primary readout and those due to PTL and PTTL for several TL dosimetry materials

Dosemeter	Primary read-out peaks	PTL peaks	PTTL peaks
LiF (TLD 100) chips	220	120 + 220	230
LiF/PTFE 0.2 mm disc	220	190	240 - 260
LiF/PTFE 0.4 mm disc	220	190	235
$CaSO_4$	160	145	165
CaF_2	170 + 250	170	190 + 280
BeO	200	180	200

Table 2
Re-assessment efficiency (expressed as a percentage of the primary read-out) as a function of re-assessment cycle for several TL dosimetry materials

Dosemeter	Re-assessment cycle 1	2	3	4	5
LiF/PTFE (0.2)	14	2	0.5	0.2	0.2
LiF/PTFE (0.4)	20	4	2	1	0.5
$CaSO_4$	1	1	1	0.5	0.5
CaF_2	0.2	< 0.01	< 0.01	< 0.01	< 0.01
BeO	10	2	1	0.5	0.2

(LiF chips were not re-assessed at an elevated temperature - see reference (1) for data)

Table 3
Detection threshold (mGy) at the 95% confidence level as a function of re-assessment cycle for several TL dosimetry materials (see ref (1) for additional results)

Material	Primary Read-out	Re-assessment cycle 1	2	3	4	5
LiF/PTFE (0.2)	2	60	400	850	1000	2000
LiF/PTFE (0.4)	0.4	60	200	450	650	850
$CaSO_4$	0.3	40	40	50	50	120
CaF_2	0.01	7	500	1000	1000	1000
BeO	0.02	2	2	10	10	15

IV. Objectives for the next reporting period:
Project completed

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

VI. Publications:

O'Hagan J B, Pearson A J, Driscoll C M H 'Multiple dose re-assessment in thermoluminescent materials', Radiation Protection Dosimetry, 17, 347-350, 1986.

Driscoll C M H, Richards D J, O'Hagan J B, Francis T M 'What's new in TLD' to be published in NRPB Radiological Protection Bulletin, 1987.

Driscoll C M H 'Dose re-assessment of LiF:Mg,Cu,P' to be published in Radiation Protection Dosimetry, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)

Prof. Dr. R. Grillmaier
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 dosimetry and radiation protection dosimetry of neutrons and photons with low pressure proportional counters.

Head(s) of project:

H. G. Menzel

Scientific staff:

K. H. Folkerts
A. Kunz
P. Pihet
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 particles) 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 yield spectra due to single primary interactions is used to determine required interaction and dosimetric data for neutrons and photons.

II. Objectives for the reporting period:

To carry out measurements with low pressure proportional counters with different wall materials for monoenergetic neutrons above 20 MeV at the Swiss Institute for Nuclear Research (SIN). To continue the investigation and optimization of the timing properties of proportional counters. To perform combined measurements of pulse height and time-of-flight spectra for neutrons and mixed radiations. To investigate possibilities to decrease the lower limit for the simulated diameter in low pressure proportional counter measurements.

III. Progress achieved:

Low pressure proportional counters (PC) with walls made of A-150 plastic (TEPC) and graphite (CPC) were used to measure ionisation yield spectra and kerma in radiation fields designed to produce monoenergetic (and polarized) neutrons in the energy range of 20-60 MeV at the Swiss Institute for Nuclear Research (SIN). The neutron beams were produced by bombarding a thin beryllium target (370 mg cm^{-2}) with protons of 29.6, 43.7 and 63.7 MeV resulting in (mean) neutron energies of 27.8, 39.7 and 60.3 MeV. The counters were filled with propane based TE-gas mixture at a pressure corresponding to a "simulated diameter" of 1 µm at a density of 1 g cm^{-3}. Cylindrical caps made of polyethylene and graphite of various thicknesses were used on the detectors in order to study the dose and charged particle build- up and to achieve charged particle equilibrium for kerma measurements.

The measurements were evaluated in terms of absorbed dose distributions in the microdosimetric quantity lineal energy and in terms of kerma. The ionisation yield spectra measured with the TEPC with and without polyethylen build- up caps and normalized to the charge of the proton beam incident on the Be-target reveal that complete build- up is achieved for α-particles and heavy recoils ($y > 150$ keV μm^{-1}) with the intrinsic wall thickness of 2.5 mm A-150. There is, however, a distinct build-up effect of fast protons ($y < 30$ keV μm^{-1}) with increasing wall thickness. The relative increase in neutron absorbed dose per monitor amounts to 30% for a 6 mm thick cap and 40% for a 13 mm cap. Comparable results were obtained at the other energies. The build-up effect in carbon seen with graphite caps is entirely due to protons from the (n,p) reaction in carbon.

Fluence and spectral fluence measurements were not available for the neutron beams used so that results cannot be given in terms of kerma factors. However, several quantitive conclusion of relevance for neutron dosimetry can be drawn from the results. The ionisation yield spectra measured with the CPC reveal the significant increase in dose contribution of protons from the (n,p) process in carbon at neutron energies above 20 MeV. In view of the importance of this and other non-elastic processes for kerma and absorbed dose in this energy range and the reverse rate of carbon and oxygen in A-150 plastic and muscle tissue the problem of tissue-equivalence in dosimetry and microdosimetry becomes very apparent. The combination of TEPC and CPC measurements ("twin counter method") has been used to evaluated gas-to-wall absorbed $r_{m,g}$ dose conversion factors for the CPC. The method tales advantage of the ionisation yield spectra and the fact that carbon has a large relative abundance of 0.776 (by weight) in A-150. Values of $r_{m,g}$ remain nearly constant (0.8 - 0.85) for TE gas filled CPC's in the energy range 15 - 60 MeV.

The kerma ratios of carbon and A-150 have been evaluated from the measurements and, together with previously obtained experimental results between 14 and 19 MeV, compared to theoretical values. Whereas there is good agreement with the data of Brenner and Dimbylow below 20 MeV, the experimental results are systematically lower for the measurements at SIN. This must be, at least partly, due to a contamination of the radiation field with neutrons of lower energies. The final measurements of kerma factors will have to include time-of-flight discrimination of the low energy neutron events.

Progress has been achieved in the understanding and optimization of the timing properties of PC. The investigation of the achievable time resolution with photons (see Progress Report 1986) was continued using pulsed beams of monoenergetic neutrons of 50 keV, 140 keV and 15 MeV at PTB, Braunschweig. These measurements provided information on the timing properties for mixed neutron gamma-ray irradiations and for the large dynamic range of pulse heights produced by neutrons. The experiments were performed using the dual parameter data acquisition system of PTB which allows detailed analysis of the correlated pulse height and time-of-flight spectra. PC's with geometrical diameters of 6.35 mm, 12.7 mm and 50 mm filled with propane or iso-butane based TE-gas mixtures or pure iso-butane were used. Similar to the results for photons also for neutrons the best time resolution was obtained with iso-butane and the smallest counter (6.35 mm counter filled with iso-butane: 30ns FWHM) . Iso-butane based TE-gas mixture was only slightly inferior. Propane based TE-gas, however, leads to comparably poor time resolution and distinct dependence of the time resolution on pulse height. The results obtained will be used for the time-of-flight discrimination of low energy neutron contamination in the high energy neutron beams at SIN.

The objectives for the reporting period have been largely achieved. No progress has been made with regard to extending PC measurements to lower simulated diameters.

IV. Objectives for the next 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 the Swiss Institute for Nuclear Research and to evaluate ionisation yield spectra, kerma and, if possible, kerma factors for different energies. To carry out measurements with graphite proportional counters for monoenergetic neutrons between 18 and 20 MeV at PTB (jointly with PTB). To compare the experimental results with theoretical data based on nuclear model calculations.

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

Physikalisch- Technische Bundesanstalt (PTB), Braunschweig
Gruppe Neutronendosimetrie (Dr. G. Dietze).
Braunschweig

National Bureau of Standards (NBS), Washington, D.C.
Center for Radiation Research (Dr. J. J. Coyne).

Universität Basel und Schweizerisches Institut für Nuklearforschung (SIN), Switzerland.

VI. Publications:

Menzel, H.G., Fast neutron and pion interaction data from low pressure proportional counter measurements, in Nuclear and Atomic Data for Radiotherapy and Related Radiobiology, Proc. of an Advisory Group Meeting organized by the International Atomic Energy Agency, Rijswijk, Sept. 1985, p. 265-284 (1987).

Prenner, D.J., Zaider, M., Coyne, J.J., Menzel, H.G. and Prael, R.E., Evaluation of nonelastic neutron cross sections in carbon above 14 MeV, Nucl. Sci. Eng. 95, 311-315 (1987).

Title of the project no.: 2

Investigations of practical aspects of employing microdosimetric counters as dose equivalent meters.

Head(s) of project:

H. G. Menzel

Scientific staff:

K. H. Folkerts
A. Kunz
P. Pihet
P. Dahmen

I. Objectives of the project:

Radiation protection dosemeters based on tissue equivalent proportional counters (TEPC) have been shown to have very good energy response for neutrons in terms of operational quantities such as $H^*(10)$ 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 calibrated neutron and mixed radiation fields and in radiation environments of practical importance. The construction will be optimized for operational health physics requirements and with regard to the instrument response.

II. Objectives for the reporting period:

To perform measurements of dose equivalent with the TEPC system in mixed radiation environments of practical importance. To participate in the second intercomparison of TEPC area monitors for low energy neutrons at the filtered beam facility of the Research and Measuring Reactor (FMRB) and at the accelerator facility of PTB, Braunschweig (coord. by EURADOS- Committee 1). To perform measurements in well defined neutron radiation fields of radiation protection and dosimetric interest. To investigate possibilities to improve the dose equivalent response of the TEPC in the intermediate and low neutron energy range.

III. Progress achieved:

The analogue electronics (linear amplifiers and high voltage supply) for our prototype TEPC area monitor "HANDI" were designed and constructed. Design criteria were low power consumption and small physical size without significant loss in performance characteristics in comparison to conventional laboratory electronic instruments. The complete prototype portable area monitor was tested in the second intercomparison of TEPC instruments at PTB (see below). In parallell work was performed using CAD- techniques in order to further miniaturize and optimize the electronics and to implement a more powerful microprocessor. The final design is expected to meet the criteria required for operational health physics instruments.

Dose equivalent measurements with TEPC systems (both the conventional laboratory system and the HANDI prototype) in mixed radiation environments of practical importance were continued. In the environments of several neutron therapy facilities in Great Britain, France, Belgium and Germany a comprehensive set of measurements was carried out. If possible comparisons were made with conventional neutron dose equivalent rate meters ("rem counters") and other instruments such as the DINEUTRON (Mourges et al., Proc. 5th Symp. Neutron Dosimetry, 397 (1984)).The results obtained show that in general neutron dose equivalent measured by the TEPC and the "rem counters" agree within 50%, the difference being largest for higher neutron energies. The differences in results were considerably larger if compared to the DINEUTRON. Measurements were also performed in working areas and storage rooms of a nuclear fuel processing plant. The diagnostic capacity of the TEPC instrument was used to analyze the radiation field and its local and temporal variation. Neutrons originate from spontaneous fission and (α,n)- reactions and are shielded by various materials of varying thickness. The spectral information was also used to assess the energy of photons. In several positions the mean photon energy was found to be less than 100 keV. The conventional microdosimetric method to determine photon dose fractions by fitting a standard photon spectrum (e.g. a ^{24}Na spectrum) is inadequate in these situations and a simple threshold method provides better results. This experience is of practical importance for the final evaluation procedures to be installed into the portable area monitor.

With these and previous measurements a comprehensive set of TEPC dose equivalent measurements in mixed fields of practical interest (physical and medical accelerators and nuclear industries) has been obtained. The investigations have demonstrated the suitability of TEPC instruments in terms of dose equivalent (rate) meters and of their diagnostic capacity. The experience gained is being included in the final design of our portable mixed radiation area monitor.

In November 1987 we participated with both, the prototype HANDI and the laboratory microdosimetry system in the second part of the intercomparison of TEPC instruments, jointly organized by EURADOS Committee 1 and PTB. At the filtered reactor beam facility of PTB the beams of thermal neutrons, 24.5 and 144 keV neutrons were used. At the accelerator facility monoenergetic neutrons 0.57, 2.5 and 14.8 MeV were available in addition to a ^{60}Co reference radiation field. The final results of this part of the intercomparison are being evaluated at present.

Theoretical and experimental studies were carried to ascertain the influence of small amounts of ^{3}He added to the counting gas on the dose equivalent response of a TEPC. The aim of this investigation is to improve the low response in the intermediate and low neutron energy range and is based on the proposal made by Psroma for ionization chambers (Proc. 5th Symp. on Neutron Dosimetry, p. 331 (1984)) . The principle of this approach is to take advantage of the moderation of low energy neutrons in the counter wall and the large cross-section of the $^{3}He(n,p)^{3}H$ reaction in which 764 keV kinetic energy is released to the charged particles. Calculations showed that a fraction of not more than 1% ^{3}He by weight should increase the response of a TEPC with walls of 3 mm thickness appreciably for neutron energies below 100 keV. For neutrons in excess of 1 MeV the influence on the dose equivalent response is expected to be negligible. Measurements at PTB with TEPC counters filled with propane based TE-gas mixture and additional 0.1, 0.5 or 1% ^{3}He were tested in the radiation fields of the thermal neutron beam, 24.5 and 144 keV neutrons (filtered reactor beams), 2.5 MeV monoenergetic neutrons and of a D_2O moderated ^{252}Cf-source. The wall thickness was varied by using polyethylene build-up caps of varying thickness. A preliminary evaluation showed principal agreement with the theoretical estimates. As an example, the addition of 0.1, 0.5 and 1.0% ^{3}He increased total dose equivalent measured by a counter with a 2.5 mm thick wall made of A-150 plastic in the thermal neutron beam by factors of approximately 3.5, 17 and 35, respectively, if compared to the pure TE- gas mixture. In the radiation field of a D_2O moderated ^{252}Cf source an increase of 30% was observed for additional 1% ^{3}He. The dose equivalent could be further increased for some of the radiations investigated by increasing the wall thickness.

The objectives for the reporting period have been fully achieved.

IV. Objectives for the next reporting period:

To carry out the final evaluation of the second part of the EURADOS intercomparison of TEPC instruments at PTB and 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 operating procedures of TEPC based area monitors. To continue radiation protection measurements with TEPC instruments in mixed radiation fields of practical interest (accelerators and nuclear industries). To continue work on the development of the portable TEPC area monitor suitable for operational health physics and to test its performance.

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

Physikalisch- Technische Bundesanstalt (PTB), Braunschweig Gruppe Neutronendosimetrie (Dr. G. Dietze) and Gruppe Metrologie der Reaktorneutronen (Dr. W. Alberts)

Universite Catholique de Louvain, Belgium (Drs.A.Wambersie and J.P.Meulders)

National Physical Laboratory, Teddington and University of Leeds, U.K. (Drs. J. Hunt and A. J. Waker)

EURADOS- CENDOS Committee 1

VI. Publications:

Menzel, H.G., Applications of microdosimetry in radiation protection, in Radiation Research, Proc. 8th Int. Congress of Radiation Research, Edinburgh, July 1987, E.M. Fielden, J.F. Fowler, J.H. Hendry, D. Scott eds., p. 345-350,(1987) (Taylor and Francis, London).

Schuhmacher, H., Alberts, W.G., Menzel, H.G., Bühler, G., Dosimetry of low energy neutrons using low pressure proportional counters, Radiat. Res. 111, 1-13 (1987).

Schuhmacher, H., Menzel, H.G., Pluge, H., Dosimetry of a bare and a D_2O moderated ^{252}Cf source using low pressure proportional counters, Radiat. Prot. Dosim. 19, 103-109 (1987)

Pihet, P., Meulders, J.P., Marquebreucq, S., Menzel, H.G., Wambersie, A., Dose equivalent determined by tissue-equivalent proportional counters in the environment of the neutron therapy facility at Louvain-la-Neuve, Brit. Journ. Radiol., 60, 311 (1987)

Menzel, H.G., A study of the National Physical Laboratory microdosimetry research programme in collaboration with the University of Leeds, NPL Report RS (EXT) 93 (1987).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no.: B16-A-025-UK

Polytechnic of the South Bank
Borough Road
GB London SE1 0AA

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

Dr. A.C. Norris
Dept of Phys.Sc.& Techn.
Polytechnic of the South Bank
Borough Road
GB London SE1 0AA

Dr. A.K.M.M. Haque
Dept of Phys.Sc.& Techn.
Polytechnic of the South Bank
Borough Road
GB London SE1 0AA

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:

Extensive analysis and comparison of dose calculation results.

Calculation of microdose spectra for alpha particles from RaA and RaC' for different lung generations and depths in tissue.

Calculation of $\bar{y}_F$, $\bar{y}_D$ and $\bar{z}_D$ and find out if they give information about radiation quality of alpha from RaA and RaC'.

Assess the radiosensitivity of those cells which are lying in the way of alpha track (including basal cells).

III. Progress achieved :

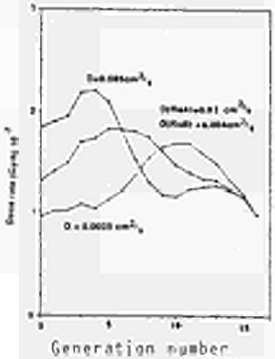
The radiation dose to the lungs due to inhalation of radon daughter products has been computed with improved data on lung models, aerosols parameters, deposition and clearance mechanisms. The effect of the following factors on calculated dose for dwellings have been considered in detail as shown below : (a) The diffusion constant of the unattached daughter products, (b) The activity median diameter (AMD) of the aerosol size distribution, (c) Flow rate of air in the respiratory tracts, (d) Lung morphometry, (e) Room ventilation rate, (f) Effect of phase of the tissue-equivalent medium on the stopping power and range of alpha particles.

Table (1)

Constants used for lung dose calculations,
Diffusion coefficient (D) for unattached RaA = 0.03 cm^2/s.
Diffusion coefficient (D) for unattached RaB = 0.004 cm^2/s.
Mucus thickness = 15 μm.
Activity Median Diameter (AMD) = 0.12 μm.
Tidal Volume (TD) = 0.75 l.
Flow rate (V) = 10.4 l/min.
Breathing rate = 15/min.
Living Conditions (LC2) of medium ventilation is assumed ($0.3h^{-1}<V<1h^{-1}$).
Activity of Rn = 23 Bq/m^3.
Alpha stopping powers and ranges are for water.
Airway diameters and lengths are for Weibel model (1964).
Dose rate is for a depth of 22 μm in tissue.

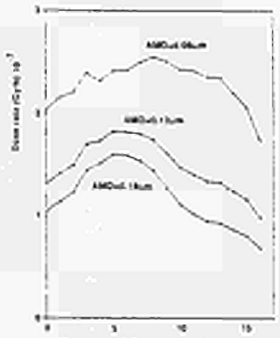
LC1: AMD=0.12 μm. f_A =10%. f_B =13%.
LC2: AMD=0.12 μm. f_A =22%. f_B =3%.
LC3: AMD=0.06 μm. f_A =15%. f_B =3%.
where f_A and f_B are the percentage of unattached RaA and RaB to total RaA and RaB respectively

(a) Effect of Diffusion Coefficient of unattached particle



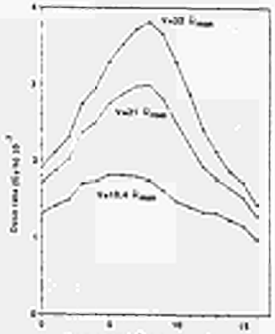
Generation number

(b) Effect of AMD



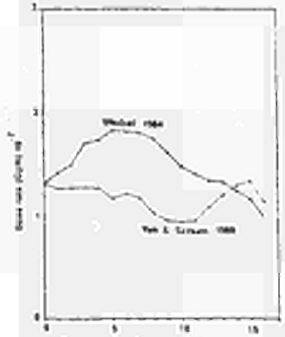
Generation number

(c) Effect of flow rate



Generation number

(d) Effect of lung morphometry



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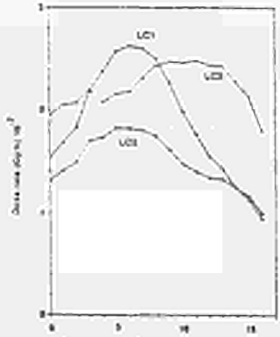
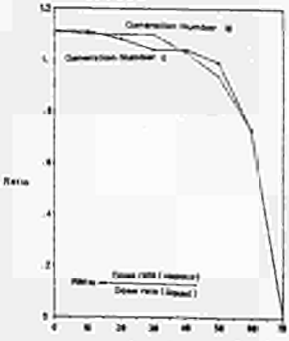
(e) effect of ventilation



Generation number

(f) Physical state effect



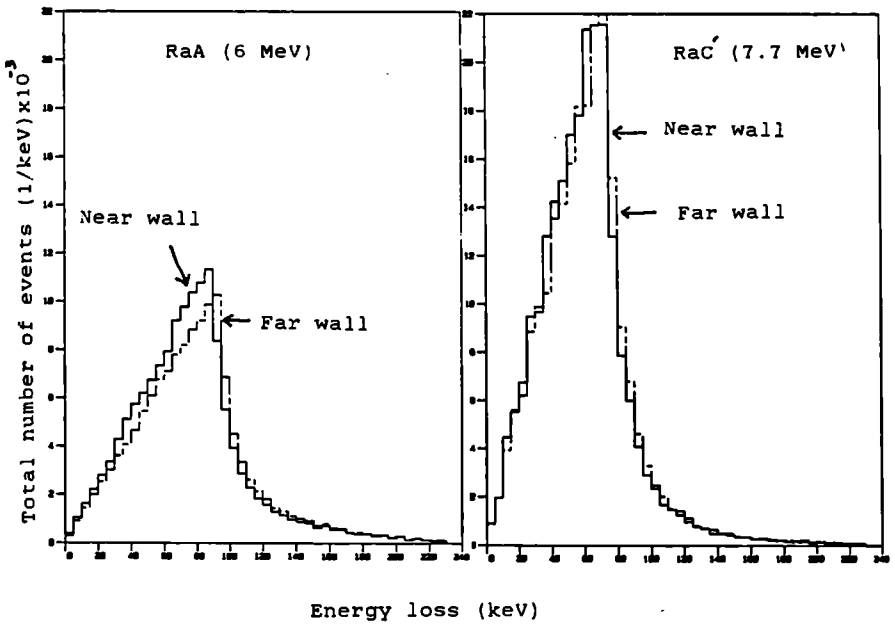
Depth in tissue (μm)

It has been found that realistic changes in values of some factors change the dose rate by a factor of 2. The annual dose in generation 5 corresponding to radon concentration of 23 Bq/m^3 (NRPB Survey) at a depth of 1 and 20 μm from the mucus-tissue interface are 4.3 and 1.8 mGy, respectively.

In order to calculate the microdose spectra for the alpha particles, a theoretical approach based on the method used by Haque (1967), taking into account the distribution of the chord length of the alpha particles striking a spherical volume (tissue) of 1 μm in diameter. Figure below shows the total number of events as a function of the energy deposited by 6 MeV (RaA) and 7.7 MeV (RaC') alpha particles. The activity of radon daughters has been assumed to line the mucus surface, the sensitive volume being at a distance of 10 μm from it.

The parameters $\bar{y}_F$ and $\bar{y}_D$ for the above spectra have been found to be 111 keV/μm and 136 keV/μm for RaA and 87 keV/μm and 107 keV/μm for RaC' respectively. $\bar{y}_F$ and $\bar{y}_D$ as a function of depth in tissue have also been calculated for RaA and RaC'.

Some chemical compounds present in mucus due to inhalation may be transferred to the tissue cells of the lung. Work is in progress to assess the radiosensitivity of those cells in the presence of chemical compounds (such as smoke).



IV. Objectives for the next 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.

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

Prof. D.M. Taylor	University of Heidelberg, Germany
Dr. A.C. James	NRPB, UK.

VI. Publications:

(1) Haque A.K.M.M. AND AL-AFFAN I.A.M. (1987). Influence of the physical state and stragglimg on the computation of the radiation dose due to radon daughters deposited in the lung. Phys. Med. Biol. 32, 1001-1005.

(2) Haque A.K.M.M. AND AL-AFFAN I.A.M. (1987). Radiation dose to the lung due to inhalation of alpha emitters, BNES Conference on Health Effects of low dose ionising radiation, London, 11-14 May, 1987.

(3) Haque A.K.M.M. AND AL-AFFAN I.A.M. (1987). Detailed cosideration of factors affecting the assessment of dose to the lung due inhalation of alpha emitters, 8th International Congress of Radiation Research, 19-24 July, 1987, Edinburgh.

(4) Haque A.K.M.M. and Al-affan I.A.M. (1987). Main factors affecting the dose calculation of lung due to inhalation of radon daughter products. (to be published) Sci. Tot. Environ. 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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:

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.:

Production of skull and chest phantoms suitable for the calibration of detectors for the measurement of Am-241, Pb-210 and Sr-90 in bone.

Head(s) of project:

F A Fry

Scientific staff:

M R Bailey, N J Dodd, M D Dorrian, G Etherington, N Green, J D Harrison

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:

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:

There has been limited progress on the original detailed objectives, primarily because of the need to redirect the staff involved onto Chernobyl-associated problems. Advantage was, however, taken of unusual opportunities to measure the distribution of bone-seeking radionuclides in man, resulting from the administration of ^{88}Y (half-life 107 days) and ^{239}Np (half-life 2.4 days) to human volunteers in experiments on radionuclide biokinetics conducted at NRPB.

In both studies the radionuclide was administered by a single intravenous injection of 0.5 ml of 0.9% sodium chloride solution to which the radionuclide had been added as the citrate at pH 7. In the first study ^{88}Y was administered to two volunteers: the first received 0.4 kBq in a pilot experiment, the second 4.0 kBq. In the second study one subject received 4.0 kBq ^{239}Np.

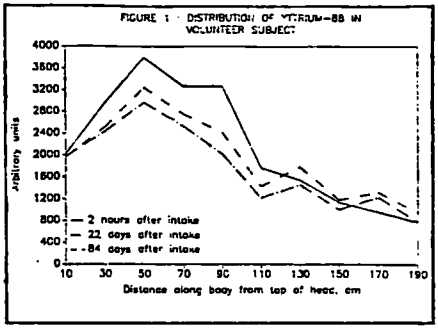
Measurements were carried out in a low-background enclosure which has steel walls 150 mm thick lined with 10 mm aged lead and 2 mm steel, and a filtered air supply. With the subject lying horizontal, longitudinal scans of the body were made using four large (150 mm diameter) cylindrical NaI(TI) detectors mounted in a vertical ring centred on the subject. The exact detector co-ordinates chosen were calculated for maximum efficiency, taking into account the size of the body. The detectors were fitted with side shields and slit collimators to reduce the contributions to the measured spectrum from activity in the body at either side of the section in view. A count time of 300 seconds was used for each of the 10 subject positions. In position 1 the top of the head was aligned with the edge of the detectors. For each subsequent position the bed on which the subject was lying was moved 200 mm along. The background-subtracted spectrum for each position was analysed using a multiple linear regression program to give an estimate of the activity present at the time of measurement, which was then decay corrected to the time of injection.

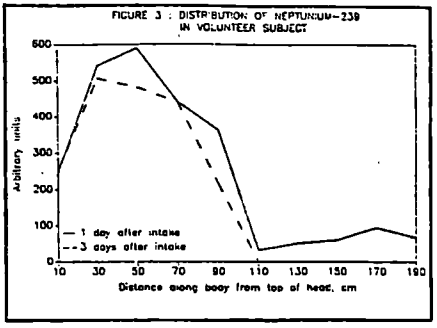
Yttrium-88

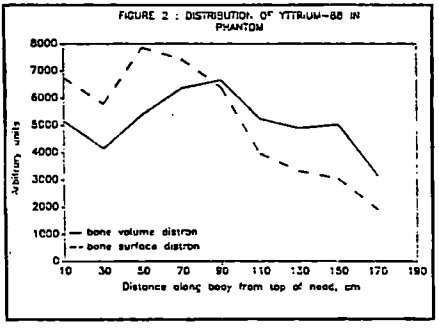
A single scan on the subject injected with 0.4 kBq ^{88}Y showed the activity present to be insufficient for this purpose. Figure 1 shows the results of scans carried out at 2 hours, 22 days and 84 days after injection on the subject who received 4 kBq. For comparison, Figure 2 shows the result of a scan carried out on a phantom constructed with ^{88}Y activity distributed according to the volume of bone in each part of the body, and the calculated response to activity distributed according to bone surface area. The latter was based on the work of Durbin and Schmidt (Health Phys. 49: 623-661, 1985). They estimated the initial distribution of americium in the human skeleton from the concentrations measured in the bones of Cynomolgous monkeys killed soon after injection of ^{241}Am, combined with the mass distribution of human skeletons. The response to activity distributed in accordance with the estimated bone surface distribution shows quite good agreement with the measurements on the subject; much better than the bone volume distribution. However, there appears to be less activity deposited within the subject's head than predicted by either model. (The additional activity at about 90 cm from the top of the head on the first scan may well be due to activity in soft tissues, and particularly the bladder. About 20% of the injected activity was excreted in urine on the first day.)

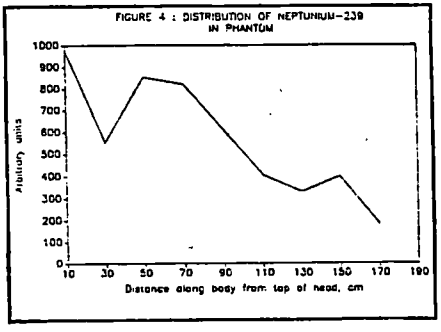
Neptunium-239

Two scans were made on the subject, at 1 and 3 days after injection, and compared with a scan of a ^{239}Np phantom containing activity distributed according to estimated bone surface area (Figures 3 and 4). However, because of the short half-life of ^{239}Np, activity in the legs could not be detected with a 300 second count after 3 days. As for ^{88}Y, there is reasonably good agreement between the subject and the bone surface model.









The measured whole body activity of yttrium-88 for the volunteer subject in Figure 1 at 2 hours, 22 days and 84 days post intake were 3.0 KBq, 2.2 KBq and 1.5 KBq respectively. In Figure 2 the yttrium-88 activity of the phantom was 11.0 KBq.

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. 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.

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

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 EPR.
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 n° : 1

Head(s) of project:

J. BARTHE - M. PETEL

Scientific staff:

R. CHUITTON

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 objective for the reporting period is the design of equipments for simultaneous EE and TL measurements and the use of beryllium oxide detectors; these detectors will replace lithium fluoride bellets which are very sensitive to thermal effects (including annealing) inside and outside the read-out system.

III. Progress achieved:

1 - **Mixed counter**

The first part of this work relates to the realization (still underway) of a double heated reader for the simultaneous counting of exoelectrons and measurement of thermoluminescence.

The most difficult problem to be solved is the spatial separation of photons emitted directly rrom the thermoluminescent sample and photons arising from electron multiplication corona effects. Two simultaneous measurement methods have been investigated:

1/ Geometrical separation: the photomultiplier only "sees" photons emitted from the surface of the sample. As the solid angle involved is very small and the sensitivity law, this solution was not retained.

2/ Electronic separation: the photomultiplier simultaneously "sees" photons emitted from the thermoluminescent sample and photons generated through electron avalancne in a zone close to the anode where the electric field is intense. Photons coming from the TL sample form a slowly varying continuous flux. Photons generated through the discharge are emitted during a very short lapse of time ranging from a few tens of nanoseconds to a few microseconds depending on operating conditions: 10^3 to 10^5 photons in the proportional counting region, 10^5 to 10^8 photons at the boundary of the proportional counting region.

The numbers of exoelectrons emitted are determined in the same way as with conventional systems. Thermoluminescence signals are determined with a circuit enabling the continuous component of the composite signal to be extracted. It is nevertheless necessary to correct this composite signal for mean deformations arising from the superpositioning of pulses due to electron avalanche. This is accomplished using the exoelectron channel to control the "coincidence" channel.

Some problems still remain: one of these problems is the persistence of the anodic sheath due to the diffusion and migration of positive ions up until the time when they are collected by the counter walls. Another

difficulty arises from changes in the instantaneous gain of the counter due to modifications in space charge that become increasingly troublesome at high counting rates.

Finally, a further difficulty has been encountered with the use of conventional LiF pellets (Harshaw) and BeO pellets (consolidated Ceramics) whose macroscopic conductivities are insufficient to ensure a good conductivity of the field at the sample.

2 - Beryllium oxide

In addition to the very low conductivity of TLD 700, other problems have arisen when attempting to use lithium fluoride (PTL 7 from Desmarquet and TLD 700 from Harshaw): after a few measurements there is incompatibility between the thermal annealings needed for TL and TSEE measurements.

Furthermore, TSEE count rates are 100 times lower than TL count rates. This results in a high threshold (about 5 mGy) which considerably limits the usefulness of such dosemeters for individual dosimetry.

In order to overcome these problems, beryllium oxide pellets (Consolidated Ceramics) have been selected. In contrast with LiF (figure 1), TSEE and TL glow curves no longer exhibit separated peaks (figure 1). It is therefore absolutely necessary to record them simultaneously with the same head.

External thermal annealing (outside the reader) is no longer necessary. However, the low electrical conductivity of BeO pellets necessitates the use of a multipoint counter the electric field geometry of which makes it less sensitive to surface charge defects.

The sensitivity of BeO pellets is quite sufficient: $3\ 10^4$ cps/mGy/cm^2 for electron emission and $2\ 10^3$ cps/mGy/cm^2 for thermoluminescence; the background signal under the same conditions corresponds to a few percent of the measured signal.

Exoemission and thermoluminescence measurement reproductivity corresponds to one reduced standard deviation at about 5%.

3 - Conclusions

The reading of beryllium oxide pellets with an appropriate reader appears to be the ideal solution for simultaneous TSEE and TL measurement of low energy electrons and photons. Phenomenological studies will be conducted as soon as a new reader head becomes available.

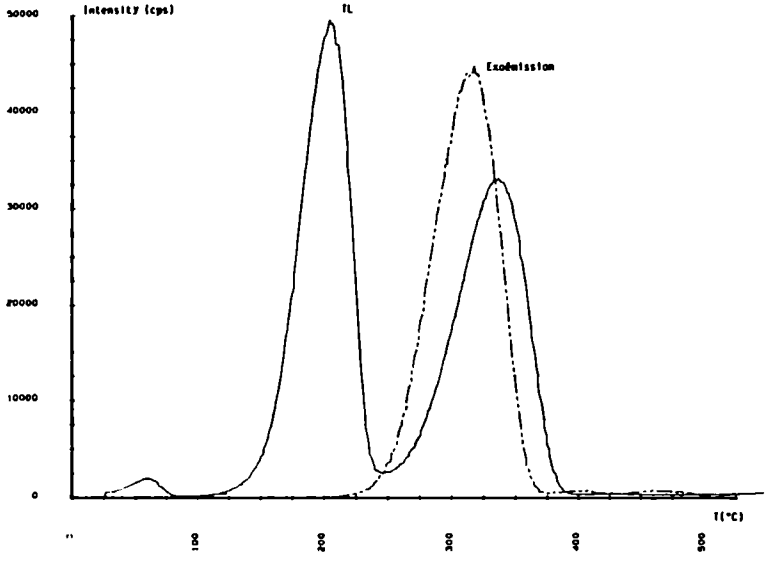


Figure 1 - EETS and TL glow curves for berylium oxide.

IV. Objectives for the next reporting period:

Termination of the development of the new TSEE reader head (multi-point).

Termination of the electronics for separating TSEE and TL signals.

Applications to measurements of the mean energies of composite β radiations.

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

Professor A. SCHARMANN
I Physikalische Institut der Universität Giessen
Heinrich, Buff-Ring 16
6300 GIESSEN - FR6

VI. Publications:

M. PETEL and J. BARTHE
"Measurement of the Mean Energy of Soft Betas using Thermoluminescence and Exoelectron Emission"
8th Inter. Conf. on Solid State Dosimetry
Rad. Prot. Dosim. 17(1/4) (1966) pp.

Title of the project n° : 2

Thermoluminescence and Electron Emission from Cotton Fabrics.

Head(s) of project:

J. BARTHE - S. LORRAIN

Scientific staff:

Ph. BLANCHARD

I. Objectives of the project:

The aim of this work is to develop a method to determine the dose received by a person accidentally irradiated with γ radiation, but not wearing a dosemeter. The clothing worn is used to estimate the dose. Clothing is made from natural or synthetic fibers very nearly equivalent to biological tissues.

Two complementary research activities are involved:

- dosimetry by low temperature thermoluminescence after phototransfer, only the clothing fabric itself being used,
- dosimetry of specially adapted textile fabrics doped with a radiothermoluminescent product. In both cases the various procedures and methodologies are defined.

II. Objectives for the reporting period:

The main objective for this reporting period is the doping of textiles fabrics with radiosensitive TL materials. This is now done because fading effects with non doped fabrics read by the Photo-Induced thermoluminescence make the method too time consuming for general use.

Phenomenology and methods are reported together with practical aspects of the work already.

III. Progress achieved:

1 - Introduction

The very low sensitivities of cotton and synthetic fabrics have led us to consider incorporating TL sensitive materials in textile fibers so as to produce fabrics with an overall enhanced radiosensitivity. Alumina (a thermoluminescent material described in a previous report) has been used for this purpose.

Textile cloths have been coated with thermoluminescent alumina in such a way as to conserve thermoluminescent properties without changing the external aspect of the fabric.

2 - Results

The following three methods have been used for readouts: low temperature photoinduced thermoluminescence, conventional thermoluminescence at positive temperatures, exoelectron emission.

- Attention is drawn to the fact that spectral emission from the A and B peaks of alumina (figure 1) is superimposed on type on the parasitic emission from the alumina support. The use of adopted filters enables the effect of this support to be reduced so as to obtain a signal free from triboluminescent effects (figure 2). However, optical fading measurements are not very encouraging: peaks A and B are attenuated by a factor of more than 50%.

After X-irradiation at room temperature, 4 peaks are observed by conventional TL: C, D, D' and E; the corresponding temperatures being: 90, 135, 169 and 255°C respectively. These peaks depend on the particular alumina used. The intensity of peak E varies linearly with air-kerma as shown in figure 3.

- Exoelectronic emission measurements are performed with a multipoint counter at a 2°C/s heating rate. Aluminized cottons exhibit after a β, γ or X irradiation a TSEE peak at 330 $\pm$ 10°C. The upper limit of the temperature region explored is 385°C because of a progressive burning of the

cotton even in a methane atmosphere. This peak is characteristic of TSEE emission from alpha alumina and does not occur with noncharged textile supports. Attention is also drawn to the fact that the charging method adopted does not lead to any parasitic triboemission as is generally the case.

- The reproducibility observed for measurements performed on several dozen different samples of charged cottons irradiated at a single dose of 0.2 Gy is about 10%; for one square centimeter surface of charged cotton fabric, we obtain at about:

Peak amplitude: 270 ± 28 counts/second
Surface area : 9847 ± 970 counts/second.

Background noise is negligible; in general tests realized up to now show such noise to be less than 10 mGy.

3 - Conclusions

Encouraging results have been obtained; however several phenomena are still not clearly understood. In particular the role of external parameters such as ambient light, storage temperature, humidity, successive washings, mechanical friction, etc.. require further investigations.

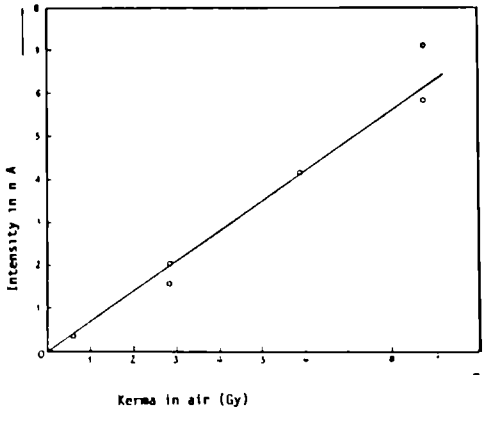


Figure 1

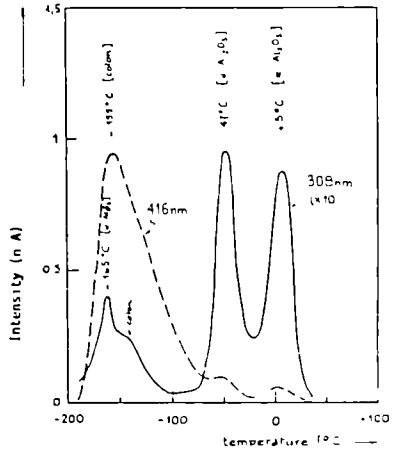


Figure 2

IV. Objectives for the next reporting period:

The main objective for 1988 is the characterization of the effects of external parameters on photo-induced thermoluminescence, thermostimulated exoemission and classical thermoluminescence response.

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-

VI. Publications:

P. IACCONI, D. LAPRAZ, P. KELLER, G. PORTAL and J. BARTHE
"Photoinduced Thermoluminescence of X-irradiated α-Al_2O_3 Dosimetric Properties"
Proc. of the VII Int. Conf. on Sol. State Dosimetry
Rad. Prot. Dosim. 17(1/4) (1986) pp. 475-478.

Title of the project n° : 3

Dosimetry on natural and synthetic fabrics by ESR.

Head(s) of project:

J. BARTHE - F. BERMANN

Scientific staff:

R. CHUITTON

I. Objectives of the project:

The general objectives of the work is to develop a method to determine the doses received by a person not wearing a dosemeter. Doses are evaluated using a method based on electron paramagnetic resonance (RPE).

The technique has the following advantages:

- it is non destructive
- the information contained can be read and reread as many times as desired
- many textile polymers give EPR signals
- textile fabrics are almost biological tissue equivalent.

II. Objectives for the reporting period:

The main objective of the work undertaken during this period has been background noise (or predose) measurements of irradiated fabrics. Generally, it will not be possible to procure fabric manufactured in the same way and having the same composition as that implicated in an accident.

In order to satisfy this objective, the thermal kinetics of the paramagnetic resonance signal were studied before and after irradiation. The results obtained are particularly encouraging for polypropylene based textiles.

III. Progress achieved:

Unirradiated fabrics exhibit a background signal due to the presence of an intrinsic concentration of free paramagnetic radicals. These radicals are generated in textile fibers as a result of natural (cotton) or synthetic (propylene) fabrication processes.

The chemical and physical treatments carried out prior to readout are described in the previous report; tne same treatments are still applied before measurements are made.

1 - Principles

KPE measurements are made on textile fabric samples after having eliminated free radicals created by irradiation. This elimination is carried out by thermal means insofar as the radicals created by irradiation are less stable than the original radicals.

In order to define and optimize (where it exists) the radio-induced radical elimination procedure, a complete study has been performed on the thermal activation energy of the radical populations.

2 - Method

Witn amorphous polymers, radical recombination is described by an equation of the 2^{nd} degree with respect to temperature. This mechanism also applies to crystalline polymers at high temperatures near to the softening point. Two main models can be used to describe the kinetic laws:

- avalanche recombination in a thermal wave
- stepwise recombination.

The first model applies to simple cases but cannot be applied to more complex cases; the second model is in more widespread use. The fundamental assumptions made are as follows:

- the existence of second order kinetics with a transient formation of a radical complex,

- a continuous distribution of radicals as a function of their activation energies,
- the existence of a capture sphere within which no recombination takes place,
- radical diffusion velocities increase with temperature.

Under these conditions, the shape of the recombination curves does not depend on the initial radical concentration for a given substance; step levels depend on temperature.

3 - Results

Figures 1, 2, 3 and 4 show the shapes of the ESR spectra as well as the evolution of the resonance amplitudes of cotton and polypropylene as a function of temperature. In the case of cotton, radical recombination is observed up to 120°C with the disappearance of two lateral resonance lines; above 170°C, the main line becomes sharper and its amplitude increases indicating that free radicals are being generated thermally. In this case, only part of the radio-induced radical population be eliminated.

In the case of polypropylene, radical recombination occurs up to 100°C accompanied by the disappearance of the radio-induced signal; above 120°C a progressive destruction occurs.

The disappearance of the ESR signal from PP created by ionizing radiation is a very interesting phenomenon, as it allows the unknown background noise of an irradiated fabric to be determined.

4 - Conclusion

The lowest detection threshold (0.5 Gy) was obtained with polypropylene fabric using a readout/heating cycle; the most accurate dose measurements were made with this material.

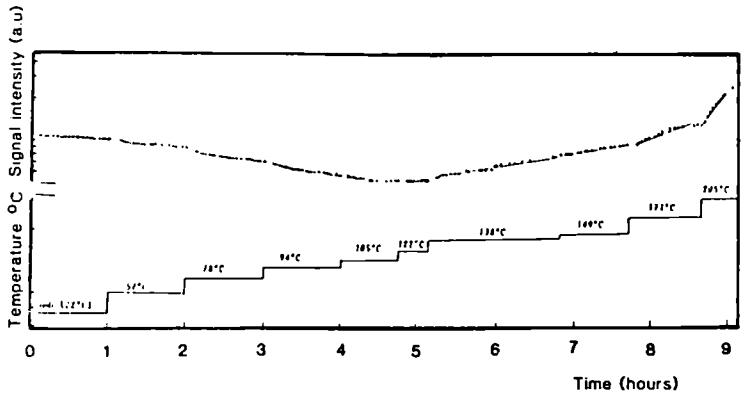


Figure 1 - Stepwise recombination for cotton fabric

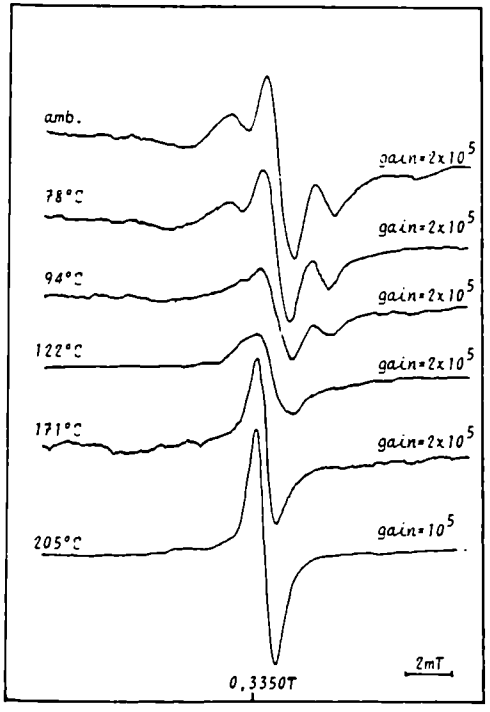


Figure 2 - ESR spectra for cotton fabric

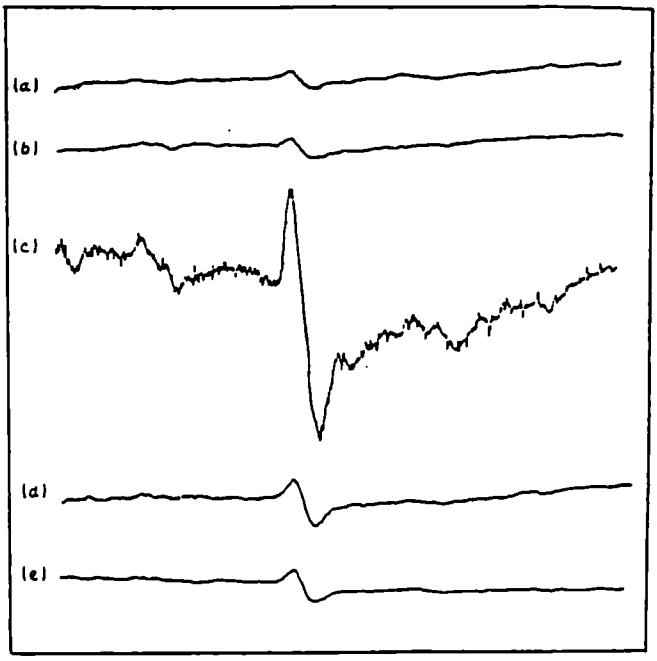


Figure 3 - ESR spectra for polypropylene

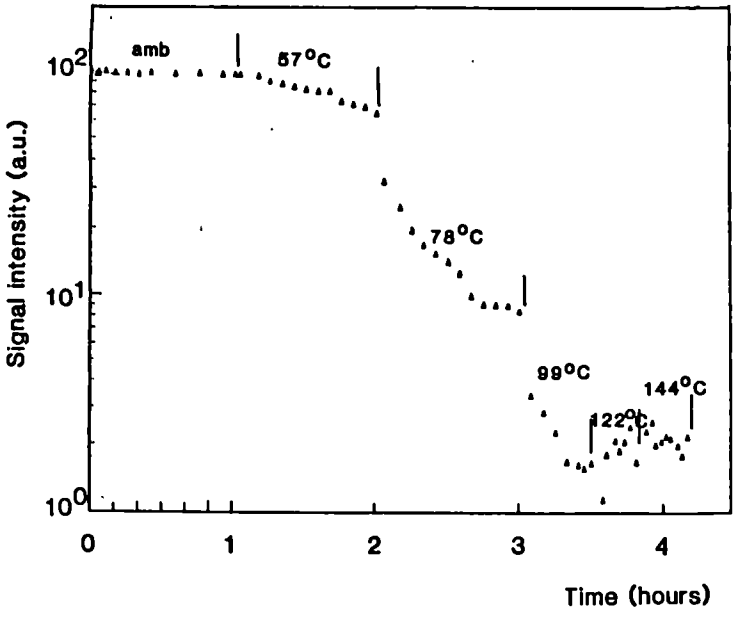


Figure 4 - Stepwise recombination for polypropylene

IV. Objectives for the next reporting period:

The main objectives to be fulfilled in 1988 is the determination of a complementary method to determine background noise from irradiated cotton samples.

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

Institut Textile de France
B.P. n°60
69132 ECULLY Cedex - France

VI. Publications:

V. KAMENOPOULOV
"Propriétés dosimétriques des fibres textiles : application à la dosimétrie par résonance paramagnétique électronique d'un accident d'irradiation gamma"
Thèse de Doctorat, n°174, Toulouse (1987), 183 pages.

V. KAMENOPOULOV, J. BARTHE, P. MICKMANI and G. PORTAL
"Accidental Gamma Irradiation Dosimetry Using Clothing"
Rad. Prot. Dos. 17 (1/4) (1986) pp. 185-188.

Title of the project n° : 4

Realization of an "operational" spectrometry unit for neutrons

Head(s) of project:

J.L. CHARTIER

Scientific staff:

F. POSNY - R. MEDIONI - J. POITREAU - M. SUEUR

I. Objectives of the project:

Study and realization of a spectrometry system for the characterization of neutron fields encountered in radioprotection.

Such fields present generally a broad and complex energy distribution, an anisotropic angular distribution and the spurious photon radiations cannot be neglected.

As also, most devices used in radiation protection give a response which is strongly dependent on neutron energy, the evaluation of dosimetric quantities needs the knowledge of the spectral distribution in a wide energy range.

For all these reasons, the detectors used must be quasi-isotropic, have a good energy resolution and be able to discriminate neutrons from photons.

To meet as closely as possible these criteria proton recoil detectors with spherical or quasi-spherical geometry have been chosen. We intend, with two spherical gas-filled proportional counters and one NE213 liquid scintillator probe, to analyze neutron fields extending from 20 keV to 20 MeV.

II. Objectives for the reporting period:

In 1987, the main objectives concerned:

- the determination of the characteristics of the two spherical counters (type SP2, manufactured by the Winfrith Laboratory, U.K.) with monoenergetic neutron beams (Van de Graaf accelerator at the CEN-Bruyères-le-Châtel)
- checking the NE213 probe response in the same beams and its efficiency calibration with the 14.7 MeV neutron beam at the BIPM
- testing the whole spectrometric system (detectors + methods + computer codes) by measurements of broad neutron spectra at the CEN Cadarache
- testing our neutron-photon discrimination method.

III. Progress achieved:

1 - Spherical counter characteristics

- N°1 is filled with hydrogen (3 bars) and covers an energy range from 20 keV to 500 keV
- N°2 is filled with methane (5 bars) and works from 120 keV to 2 MeV.

In both, traces of ^{3}He were added as calibration gas.

With monoenergetic neutrons from 60 keV to 2 MeV, we have determined:
- the experimental response functions (10 energies for n°2, only 3 for n°1)
- the efficiency curve for n°2 (linear variation from 95% at 120 keV to 65% at 2 MeV)
- the variation of the energetic resolution for n°2 (between 20% and 10% in the 120-400 keV range; less than 10% above 500 keV).

We have now to confirm the results for n°2 and to continue the experiments for n°1 (next measurements at Bruyères-le-Châtel in January 1988).

The knowledge of these experimental parameters is essential to justify the simplifying hypotheses of the unfolding code (based on SPEC4).

2 - NE 213 probe

The same beams (for energies between 1 to 2.4 MeV) have allowed the control of some characteristics of the probe.

Some discrepancies in the results led us to verify the energy and efficiency calibration with 14.7 MeV neutrons at the BIPM. A defect in the electronic system was observed.

Complementary measurements with a calibrated Am-Be source confirm our previous conclusions. Modifications in the unfolding code (Tom's code) are in progress to input the experimental values of the efficiency, meanwhile a more accurate code is under elaboration and comparison with the Tom's one.

3 - Spectrometry of wide spectra

The test of the whole Spectrometry System (3 detectors + 2 electronic lines + 2 unfolding codes) was achieved in wide spectra at the CEN-Cadarache (from 20 keV to 15 MeV). The results are encouraging but show that other measurements must be done.

4 - Neutron-photon discrimination

The spectrum of a calibrated Am-Li source was measured with the methane-filled counter. Results are in progress.

In conclusion, the good quality of the SP2 proportional counters have allowed us to make progress in the realization of the operational spectrometry system.

IV. Objectives for the next reporting period:

Next year programs will be devoted on unfolding codes to achieve more accurate results and evaluate the uncertainties.

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

VI. Publications:

Two papers have been presented at the Sixth Symposium on Neutron Dosimetry (Munich - October 1987).

They will lead to a publication in "Radiation Protection Dosimetry" under the title: Experimental simulation and characterization of neutron spectra for calibrating radiation protection devices".

Title of the project n° : 5

Study and realization of an individual neutron dosemeter based on photographic emulsions

Head(s) of project:

G. PORTAL

Scientific staff:

C. HEILMANN, Ph. BLANCHARD

I. Objectives of the project:

Replace 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:

Study of a method to discriminate between the signal due to neutron interactions and the signal due to photon radiations.

Determination of minimum detectable doses.

III. Progress achieved:

Studies conducted in 1987 have shown that it is possible to use neutron activation to determine the residual silver present in emulsions after appropriate processing.

This method has been applied to neutron detection. We have studied the principles of an individual dosemeter consisting of various nuclear emulsions and a hydrogen converter. The protons generated in (n,p) reactions on the hydrogen nuclei of the converter are detected by this method in the developed emulsion. The difficulties involved in the technique led us to study ways of reducing background signals from unused emulsions and to the development of discriminating techniques to minimize the effect of electromagnetic gamma radiation on measurements. Work essentially involved determining dose thresholds. It was shown that with current know-how that this threshold is about 1 mGy, a level that is sufficient for certain physical studies but insufficient to determine doses received by personnel. Work is being continued in 1988 in order to further diminish the effects of electromagnetic radiation so as to reduce this threshold.

IV. Objectives for the next reporting period:

Reduction of effects due to photon radiation.
Decrease of the minimum detectable dose.

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:

Thesis of University: Strasbourg
C. Heilmann

Title of the project n° : 6

Area and Individual Dosimetry with proportional counters

Head(s) of project:

J. BARTHE - M. PETEL

Scientific staff:

R. CHUITTON - J.C. CHAPUIS

I. Objectives of the project:

The objectives of this work are multipurpose:

1/ to realize a new tissue equivalent plastic conducting material to replace the out of manufacture american product

2/ to realize a large tissue equivalent proportional counter (TEPC) which can be employed inside a rem-meter

3/ to optimize by calculations and experiments the ionization coefficient of the gaseous mixture, and, in consequence, the gaseous gain

4/ to realize a dosemeter-individual rate meter for equivalent dose measurements.

II. Objectives for the reporting period:

The work accomplished this year (1987) relates essentially to developing and experimentally verifying a mathematical model to solve Boltzmann's equation at thermodynamic equilibrium and the establishment of the corresponding computer programs; special attention has been given to the cross-sections used for high E/P values [10^3 to 10^4 townsends].

Although different gases have been considered, calculations and experimental measurements have only been done up today for nitrogen and methane.

III. Progress achieved:

The work presented in this report relates to modelizing the operation of a proportional counter through finding solutions to the continuity equations describing the spatio-temporal behavior of electrons and ions.

The modelized counter is of a small size corresponding to the individual dosemeters studied. This simulation enables the evolution in time of phenomena resulting from an irradiation of the counter to be monitored, which leads in the present case, to the production of electrons in the gas cavity.

1 - Geometry

The structure of the modelized counter corresponds to a cylindrical wire model. The anode (wire) is raised up to a potential of between 0 and 10^3 volts; the cathode is at a ground potential.

The dimensions of the counter are as follows:
- anode radius : 25 μm
- cathode radius : 1 cm
- height of cylinder: 1 cm.

The cavity is filled with gas (N_2 or CH_4) at a pressure of 40 torrs. The source particles, the electrons, are introduced into the gas with a uniform volumic distribution inside a coaxial cylindrical sleeve 1 mm thick located 1 mm from the cathode.

In practice, electrons are generated by ionizing particles extracted from the cathode by the incident radiation. In the region around the cathode, the electrons generated experience little multiplication; the configuration chosen most closely simulates the distribution due to a charged secondary particle traversing the counter as a chord near to the anode, angular symmetry being taken into account.

2 - Operating hypotheses

The electric field leading to charge displacement increases strongly as the anode is approached; near to the anode the electrons possess sufficient energy to generate ions in collisions with gas molecules. These collisions (characterized by the ionization coefficient α) between electrons and neutral molecules lead to the generation of positive ions and new electrons.

It is assumed that during the preliminary period there are no electron attachments to the neutral molecules. The recombination of electrons and positive ions is also neglected. Modifications in the local electric field through space charge effects are considered as well as particle diffusion which tends to homogenize space densities. It is also assumed that there are no secondary particle emissions on the electrodes. The only charged particle populations taken into account in the calculations are those of electrons and positive ions.

Ionization and diffusion coefficients are only functions of the position of the point considered in the cylindrical volume and to a first approximation depend only on radius; displacements and collisions occur along such radii; correlations do not exist between particles circulating on different radii.

3 - Results

The program developed enables the densities of particles at a given instant in the interelectrode space to be determined. As shown in figure 1, an electron packet introduced near to the cathode first diffused in the gas, then spreads out towards the anode. A high concentration of positive ions is observed near the anode leading to a lot of multiplication: the densities in the vicinity of the wire are very high. After about 70 ns, all the electrons are collected, the number of positive ions remains constant but the packet diffuses and moves slowly towards the cathode for collection.

Figure 2 shows the quantity of charge collected as a function of time. A rapid collection of electrons is observed together with a slower collection of positive ions.

4 - Conclusions

Comparisons between calculated and experimental results, in particular for N_2 and CH_4, show good agreement for gaseous gains (less than 20% difference between values).

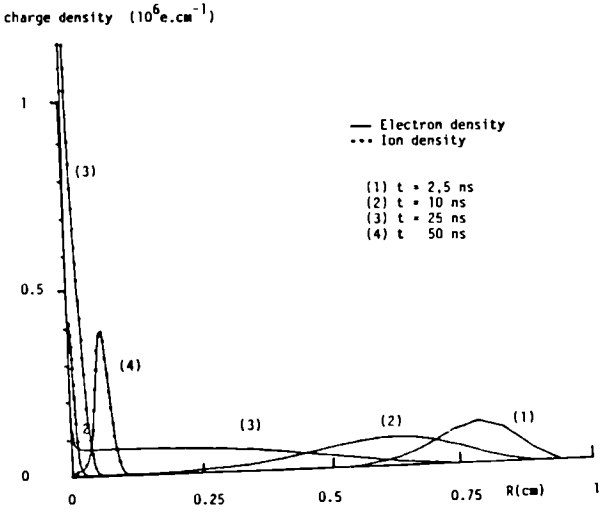


Figure 1 : Electron and ion densities

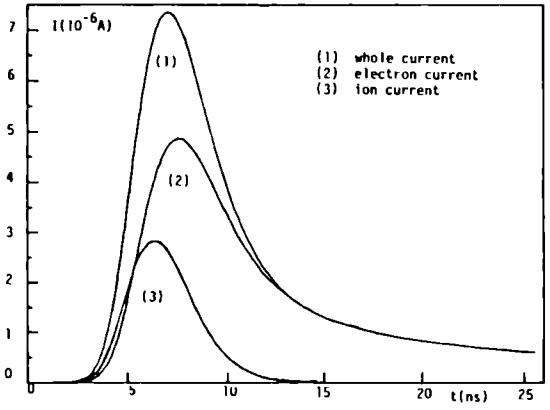


Figure 2 : Current pulse

IV. Objectives for the next reporting period:

The main work envisaged for 1988 still related to calculations and experimental measurements on the gazeous gain for a counter geometry close to the optimum solution end with a gazeous mixture compromize between highest gain and sensitivity and good tissue equivalence.

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

Groupe de Recherche sur les Décharges de faible intensité :
Centre de Physique Atomique, Université Paul Sabatier,
31062 TOULOUSE Cedex - France -

VI. Publications:

None for 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

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 interactive opto-electronic analysing system has been further developed in order to improve the experimental track feature data base, notably by the development of specific codes for automated data acquisition and treatment. In addition, theoretical ionization data have been derived by means of the Double-Differential Cross-Section Mixed Treatment (DDCS-MT) in the case of molecules of biological interest ionized by protons. A computer-assisted method has been developed which allows multidimensional graphical representations of microdosimetric quantities obtained by means of available theoretical, empirical or semi-empirical expressions.

III. Progress achieved:

The quantitative evaluation of heavy charged particle (hcp) track parameters constitutes an essential contribution to the edification of a realistic hcp track model code. Therefore, experimental as well as theoretical studies related to various track structure parameters have been continued.

1. Computer-assisted track structure analysis

Let us recall that in order to analyse the ionizing interactions between the incoming charged particle and the constituents of the absorbing medium, i.e. nuclear emulsion, modular polyvalent opto-electronic image analysis systems which basically consist of three constantly interacting elements, namely the human operator, the hardware constituting the assembly and the associated software are actually developed and improved. The possibilities of the assembly have been extended, notably by the adjunction of various modules allowing a higher automatization of the data collection. Elaborated data acquisiton and treatment codes have been and are designed for handling the different tasks related to the track structure analysis. These programs have been splitted up into three main functional component parts : a) conversational-, b) processing-, and c) control modules. These codes allow, among others, to perform measurements of the various track parameters (Fig.1). Specific evaluation softwares have been designed to analyse the input track data. Due to the flexible structure of the software, the codes can be easily modified or completed. These interactive procedures are actually applied to investigations concerning the distribution of "intergranular gaps" observed along α-particle trajectories recorded in emulsions of various compositions, as well as to hcp range measurements.

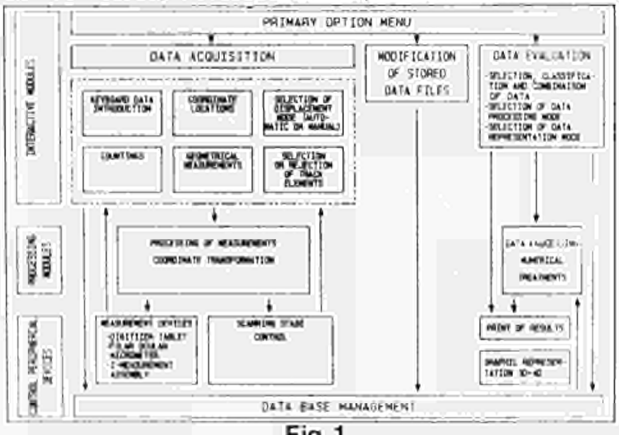


Fig.1

2. Ionization of molecules by heavy charged particles.

The primary ionization cross-sections constitute one of the main elements of the modular track structure simulation code. The Double-Diffe-

rential Ionization Cross-Section Mixed Treatment (DDCS-MT) has therefore been applied to organic molecules, notably those constituting the tissue-equivalent gases. Thus, double-differential and single-differential ionization cross-sections (DDCS and SDCS, respectively) have been calculated for methane (CH_4), by taking into account the molecular structure of this target following the principles already applied previously to water vapour. For incoming proton energies E ranging from 0.25- to 2.0 MeV and ejection energies T above ∿10 eV, the agreement between the calculated and the corresponding measurements was satisfactory.

Since the validity of a particle track simulation depends directly upon the input data, it is interesting to intercompare various sets of such data prior to their introduction in a code. Therefore a computer-assisted method has been developed which allows to represent track parameters as multi-dimensional graphical drawings, eventually in colours. This method is illustrated in Fig.2, representing the product TxSDCS as a function of E and T. The SDCS have been evaluated by means of the DDCS-MT, the Binary Encounter Approximation (DDCS-BEA, integrated over the ejection angle θ) and the Modified Rutherford formula (MR). On these pictures, it can be seen that the energy deposition pattern corresponding to the three approaches are strikingly different ; these differences would be difficult to apprehend, at least in certain cases, by means of more conventional methods (e.g. purely numerical outputs or two-dimensional graphics).

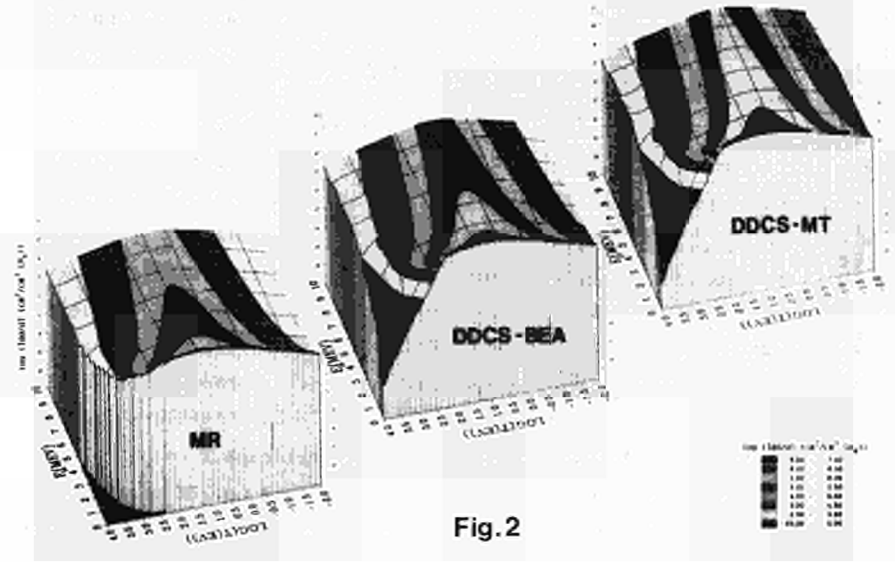


Fig.2

IV Objectives for the next reporting period:

Further experimental investigations will be carried out on the determination of track parameters, along trajectories of hcp's recorded in nuclear emulsions. Programs will be worked out in order to perform accurate data acquisition and treatment of these track features. In the framework of the edification of a modular track structure model, it is foreseen to develop semi-empirical formulas of the differential and integrated electron elastic scattering cross-sections. A particular attention will be paid to the possibility to cover a wide energy range (especially towards the low energies) as well as a large variety of molecules. Intercomparisons between track parameter data obtained by various approaches will be continued by means of the computer-assisted multi-dimensional graphical representation method.

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

- Laboratoire "Rayonnements et Structures" - Université de Metz (Prof. C. TAVARD).

- Fom-Institute for Atomic and Molecular Physiscs, Amsterdam (The Netherlands) (Dr. J.B. SANDERS).

VI Publications:

- R.V. RECHENMANN, B. SENGER and Ph. SCHALK, Computer-assisted multi-dimensional representations of ionisation cross-sections for medium energy α-particles traversing tissuelike media. Innov. Tech. Biol. Med. 8, 391 (1987).
- E. WITTENDORP-RECHENMANN, J.-L. VONES H, R.V. RECHENMANN, and C. KLEIN-SOYER, J.-P. CAZENAVE, Development of a computer-assisted methodology combined with a specific autoradiographic procedure. Application to the study of human endothelial cell regeneration. Submitted to Innov. Tech. Biol. Med.
- E. WITTENDORP-RECHENMANN, J.-L. VONESCH, V. KOZIEL-VIGNERON and R.V. RECHENMANN, Modular intera tive opto-electronic system for track structure analysis. VIth Symp. on Neutron Dosim., Munich (1987).
- 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. VIth Symp. on Neutron Dosim., Munich (1987).

Communications

- B. SENGER, E. WITTEN ORP-RECHENMANN et R.V. RECHENMANN. Sections efficaces doublement différentielles d'ionisation par particules chargées lourdes. Actes du Colloque (e,2e) (R°P n°784 NRS , Paris (décembre 1986) ; ed. Cl. Tavard, Metz (1987).
- S. FALK, B. SENGER et R.V. RE'HENMANN, Diffusion des électrons produits au cours de l'ionisation de molécules par des particules chargées lourdes. To be published in the Actes du olloque (e,2e) (R°P n°784 CNRS), Paris (1987).

Diplôme d'Etudes Approfondies (D.E.A.) : spécialité "Physique Radiologique".

DJAMEL ADDI. "Relation parcours-énergie des particules α d'énergies moyennes (E<13.6 MeV) dans des milieux tissulaires. as des émulsions ionographiques à 68% et 80% de composante NOH en volume". Iniversité ouis Pasteur, Strasbourg et niversité Paul Sabatier, Toulouse 198?).

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

I. Physikalisches Institut
Justus-Liebig Universität Giessen
Heinrich-Buff-Ring 16
D-6300 Giessen

Contract no.: BI6-A-022-D

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

Prof. Dr. A. Scharmann
I. Physikalisches Institut
Justus-Liebig-Universität
Heinrich-Buff-Ring 16
D-6300 Giessen

Telephone number: 641-702.27.10

Title of the research contract:

Beta-ray individual dosimetry using exoelectron emission.

List of projects:

1. Beta-ray individual dosimetry using exoelectron emission.

Title of the project no.: B16-022-D

Beta-ray individual dosimetry using exoelectron emission

Head(s) of project:

Prof. Dr. Drs. h.c. A. Scharmann

Scientific staff:

2 scientific assistants
1 technical assistant

I. Objectives of the project:

Development of a finger ring dosemeter, discrimination between skin and depth doses, testing of a novel conducting polycarbonate foil as a dosemeter cover and making available of BeO thin film dosemeters for the laboratory at Fontenay-aux-Roses.

II. Objectives for the reporting period:

III. Progress achieved:

1. Introduction

Thermally stimulated exoelectron emission (TSEE) is an effect whose mechanism is closely related to thermoluminescence. During exposure to ionizing radiation electron traps in the energy gap of insulators are occupied. Without transfer of additional energy this state can be conserved over a long period. Upon thermal stimulation of the solid trapped electrons are released into the conduction band. Those in the vicinity of the surface will be able to leave the solid and to be detected as exoelectrons if their energy exceeds the electron affinity. The TSEE intensity is peaking at characteristic temperatures and the area under the TSEE maxima provides a measure of the absorbed dose.

Exoelectrons are emitted from an escape depth of less than about 15 nm. In spite of this surface behaviour TSEE dosemeters have a sensitivity for penetrating radiation which is similar to conventional solid state bulk methods like for instance TLD. The density of the dose information in a 15 nm surface layer is therefore extremly high. For this reason TSEE offers favourable properties for the detection of weakly penetrating radiation. It is suited for the detection of skin doses and depth dose distributions in mixed fields of gamma and low energy beta radiation.

In a joint research project this application has been tested together with the institute of the Commissiariat a l'Energie Atomique in Fontenay-aux-Roses (France). Moreover an attempt was made to develop a finger ring dosemeter based on TSEE. The investigations were carried out with BeO thin film detectors whose general dosimetric properties are described in (1).

2. Detectors

The detectors were prepared by the Battelle Institute in Frankfurt in the following manner:

- evaporation of a 75 nm Be film onto graphite discs,
- subsequent heat treatment at 1200 - 1300 °C in wet nitrogen (10 mbar water vapour) for oxidation and sensitization.

Detectors of two different sizes were available:

- type 1 had an outer diameter of 13 mm and was designed for whole body dosimetry,
- type 2 had an outer diameter of 4.5 mm for applications in finger rings.

From previous measurements is known that such detectors fairly well fulfill the Bragg-Gray conditions when irradiated with photons. The major proportion of the exoelectron emitting traps is not occupied by secondary electrons created in the BeO layer or the graphite substrate but by elctrons impinging from outside. For this reason the tissue equivalence of the dosemeter is mainly depending on the composition of the covering material. Throughout all the investigations of the present research the detectors were covered with Macrofol, a black, graphitized polycarbonate foil. Due to its electric conductivity this foil prevents electrostatic charging in front of the BeO layer. Additionally optical fading is avoided which, however, would only be weak. With respect to its effective atomic number Macrofol behaves like carbon.

BeO thin film detectors have no uniform sensitivity for ionizing radition, even those of the same production cycle. Therefore an individual calibration of each detector was necessary. The sensitivities varied by a factor of about 2 for detectors of the same size. Since the TSEE is related to the surface properties of the exoelectron emitting solid, it can strongly be affected by surface contaminations. The dose response of almost all formerly discussed TSEE dosemeters was influenced by exposure to humiditiy. BeO thin film detectors behave comparatively more stable. They show no significant effect when stored over several weeks under saturated methanol or water vapour. However, the response may vary, if they are dropped into water. The size of this effect is not uniform. Therefore all detectors were submitted to a test procedure prior to their further use. It consisted of the following steps:

a) pre-annealing in the read-out equipment from room temperature to 600 °C without previous exposure to ionizing radiation
b) 3 subsequent ^{60}Co exposure (0.33 mSv) and TSEE read-out cycles
c) storage of the unirradiated detectors in distilled water over 24 hours
d) drying over 24 h hours in air at room temperature
e) steps a) and b)
f) storage of irradiated detectors in distilled water over 24 hours
g) drying over 24 h hours in air at room temperature
h) dose read-out
i) step b).

This test procedure enabled the preselection of detectors whose dose response was reproducible even under the worst case of a strong exposure to humidity. A typical result for such detectors is shown in Fig. 1. During the further dose measurements the detectors could be re-used after each readout without any additional treatment. In spite of the positive results demonstrated in Fig. 1 the BeO layer must be protected against contact with undistilled water, because calcareous deposits would diminish the exoelectron emission probability.

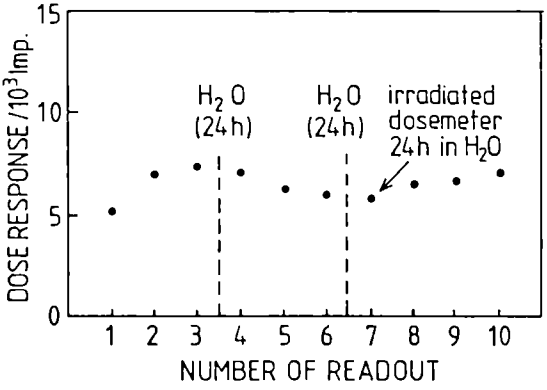


Fig. 1: Reproducibility of the dose response of a BeO thin detector after exposure to distilled water (0.33 mSv ^{60}Co)

3. Dose readout

Since BeO thin film dosemeters cover a linear dose range of more than 6 orders of magnitude a proportional counter was applied which enabled a sufficient pulse resolution for high dose readouts. It consisted of a cylindrical counting tube. The samples were heated-up in the counting volume. In order to establish a constant gas amplification of 10^5 temperature variations of the counting gas were avoided by stabilizing the tube and the gas on a temperature of 35 °C. Fluctuations of the gas pressure were compensated by equivalent variation of the counting voltage. The counting gas was methane.

4. Skin and depth dose determinations

BeO thin film dosemeters were covered with Makrofol sheets of a thickness between 1.9 - 19.2 mg/cm² and exposed to the beta radiation from the radionuclides ^{147}Pm, ^{204}Tl and $^{90}Sr+^{90}Y$ of a Buchler beta ray secondary standard at the CEA institute in Fontenay-aux-Roses. The dosemeters were placed on a plexiglass support mounted on a phantom. For reference irradiations with a ^{60}Co source they were covered with 10 mm plexiglass. Both beta and photon irradiations were carried out with a constant cavity absorbed dose of 2 mGy referred to the irradiation position at the surface of the cover in front of the dosemeters.

The response after irradiation with beta rays has been normalized to the TSEE following ^{60}Co irradiation. For ^{204}Tl and ^{147}Pm, Fig. 2a displays the relationship between foil thickness and the TSEE sensitivities obtained in this manner. The results for $^{90}Sr+^{90}Y$ are shown in Fig. 2b. For the same irradiation arrangements the dependence of absorbed doses in soft tissue on depth in a tissue equivalent semi-infinite phantom is also plotted in Fig. 2a and Fig. 2b. The data needed for these plots have been taken from the PTB calibration reports of the Buchler beta ray secondary standard.

According to these reports the absorbed dose $D_g(d)$ in a depth d can be described by

$$D_g(d) = D_c \cdot k_D \cdot T(d)$$

where D_c = cavity absorbed dose at d = 0, generated in an air filled cavity under Bragg-Gray conditions

k_D = mass stopping power ratio of tissue and air averaged over the spectral beta-ray flux

$T(d) = D_c(d)/D_c(d=0)$ (transmission factor)

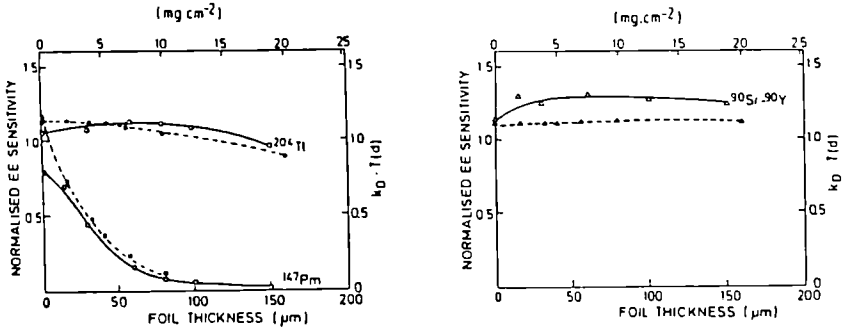


Fig. 2a: Open symbols, full line:
Dependence of the TSEE sensitivity of Makrofol covered BeO thin film dosemeters for beta rays from ^{204}Tl and ^{147}Pm on foil thickness. All values are normalized to the TSEE response after exposure to ^{60}Co photons and referring to a constant cavity absorbed dose at the outer surface of the covering foils.
Closed symbols, dashed line:
Normalized depth dose in soft tissue ($k_D \cdot T(d)$).

Fig. 2b: Results according to Fig. 2a for $^{90}Sr+^{90}Y$.

The absorbed dose $D_g(0)$ in tissue at depth d = 0 is given by

$$D_g(0) = D_c \cdot k_D.$$

Since all sensitivities depicted in Fig. 2 are referring to constant values of D_c only the products of $k_D \cdot T(d)$ have been plotted.

The normalized TSEE sensitivity of BeO thin film dosemeters covered with Makrofol sheets is related in almost the same manner to foil thickness as the product k_D T(d) to the depth in soft tissue. An air gap of 0.5 mm between the covering polycarbonate foil and the BeO surface of the dosemeter or differences of the substrate thickness in the range between 1 - 3 mm had no influence on the measured TSEE results.

The electron/photon sensitivity ratio of the dosemeters is close to 1, as indicated by Fig. 2a and 2b, because all plotted TSEE responses are normalized to ^{60}Co photon radiation. Former investigations [7] revealed a linear dose range from about 10 μGy to 10 Gy.

5. Participation in the 1986 CEC Beta Intercomparison

The CEA institute in Fontenay-aux-Roses and the I. Physikalisches Institut of the university of Giessen participated with 46 BeO thin film detectors in the CEC beta dosimeter intercomparison. It was intented to carry out the test in the following manner:

1. In Giessen:
   Preselection of BeO thin film detectors.
   Encapsulation in specific TSEE batches.
   Precalibration with ^{60}Co.
   Mailing of the dosemeters to the CEC irradiation laboratories.

2. In Fontenay-aux-Roses:
   Readout of the doses given by the irradiation laboratories.
   Calibration of the response for beta rays with ^{90}Sr.

3. In Giessen:
   Additional calibration with ^{60}Co.
   (This additional measurement cycle should ensure that the TSEE properties of the dosemeters remained constant over the duration of the test.)

According to these intentions BeO thin film detectors of an outer diameter of 13 mm were provided with batches in Giessen and covered with Makrofol sheets of a thickness of 60 μm according to 7 mg.cm^{-2}. All preselected

46 dosemeters were exposed to ^{60}Co and readout in Giessen three times with the above mentioned proportional counter. Then they were sent to the CEC irradiation laboratories.

When they came back from there they were readout and calibrated with ^{90}Sr in Fontenay-aux-Roses. Here two different readout equipments were available, a mono-needle counter and a recently developed multi-needle counter. The latter system is the more advanced. However, until 1986 it was not sufficiently tested for routine applications. For this reason the older mono-needle Geiger-Müller equipment was employed for the CEC intercomparison, whose reliability has been proved during numerous previous applications.

In former TSEE experiments with beta-irradiated BeO thin films the ratio of the sensitivities beta/gamma was close to 1. However, when the calibration results of the ^{60}Co and ^{90}Sr irradiations in Giessen and Fontenay-aux-Roses were compared it turned out that they differed for the majority of the dosemeters in an unexpected manner, although the TSEE characteristics of the detectors had not changed. For this reason the ^{90}Sr calibration in Fontenay-aux-Roses was repeated with a Buchler secondary standard. Afterwards all dosemeters were irradiated again and sent to Giessen for an additional readout. The readout measurements in Giessen gave a better agreement between beta and gamma calibration.

Additional experiments showed that the readout problems for beta-rayed dosemeters in Fontenay-aux-Roses were caused by charging-up of the BeO thin films during the TSEE measurements. Its amount was depending on the insulating character of the films and was not uniform for all detectors. Since mono-needle Geiger-Müller counters are operating at a significantly higher gas amplification than proportional counters they produce more positive ions and stronger charging effects. Obviously the TSEE dosemeters used for the CEC intercomparison charged up more than formerly used samples.

Under these circumstances the calibration results obtained in Giessen were considered as more reliable and used to calculate the CEC intercomparison doses by applying them for the original readout results in Fontenay-aux-Roses. After these doses had been reported to the CEC the dosemeters which showed the strongest and the lowest charging effects were used for a direct comparison of the recently developed multi-needle counter of the CEA laboratory in Fontenay-aux-Roses and the proportional-counter in Giessen. The experiments were carried out in Giessen. Both instruments had to read-out the same set of 8 dosemeters which were irradiated with a ^{60}Co source in one cycle and with ^{90}Sr in a second cycle. The whole procedure was repeated three times in order to test the reproducibility. For each series of three similar measurements the standard deviation of the TSEE response was below 10 % and good agreement between proportional and multi-needle counter was obtained.

From these results can be concluded that both instruments are also suited for TSEE measurements on highly insulating samples. The TSEE data of the CEC intercomparison would therefore probably have been better, if they were not obtained with an obviously less reliable mono-needle counter. These data are summarized in Fig. 3 which has been taken from the CEC report on the results of the intercomparison (2). Its ordinate values give the ratio of the measured to the true dose. Although the results are beyond the possibilities of BeO thin films because of the reasons mentioned above, it can be seen that the deviations between true and measured dose are not dependent on the beta energies.

Development of a finger ring dosemeter

For the application in finger rings 300 dosemeters with a diameter of 4.5 mm (type 2) were ordered from the Battelle Institute in Frankfurt. Like type 1 detectors of 13 mm diameter they exhibit TSEE maxima at about 270 and 480 °C, but with a different ratio of the intensities. The reproducibility of the dose response is the same. If both TSEE maxima are taken for dose readout, the sensitivity differs from type 1 detectors by a

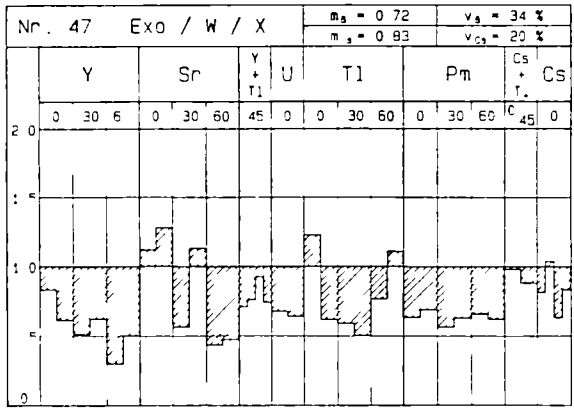


Fig. 3: Intercomparison results obtained with BeO thin film detectors for exposures to various radionuclides (2)

factor of about 4.5 according to the smaller BeO area. The lower detection limit for gamma or beta rays is in the range of about 0.14 - 0.18 mSv.

The diameter of 4.5 mm enabled the application of finger rings which are used for LiF thermoluminescence detectors by the CEA institute in Fontenay-aux-Roses. In this device the detectors are enclosed by a heat shrinkable sleeve consisting of PVC which has an unshrunk thickness of 0.07 mm. It turned out that is was difficult to get the enclosure waterproof for BeO thin film dosemeters. For this reason an improved finger ring construction was necessary. The measurements with this new construction are not finished until now.

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1. W. Kriegseis, A. Scharmann, W. Senger, A. Weiss, C.U. Wieters and B. Woerner: Dosimetric Properties of Exoelectron Emitting BeO Thin Film Detectors. Atomkernenergie-Kerntechnik 46, 264-271 (1985)

2. J. Böhm: 1986 Beta Intercomparison, CEC-Report (1987)

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

Institut de Protection et de Sûreté Nucleaire
Commissariat à l'Energie Atomique
BP No. 6
F-92260 Fontenay-aux-Roses
France

V. Publications:

1. W. Kriegseis, M. Petel, A. Scharmann and C.U. Wieters: Potentials of TSEE for Beta Ray Dosimetry. Radiat. Prot. Dosim. 14, 151-155 (1986)

2. W. Kriegseis, M. Petel, G. Portal, A. Scharmann and C.U. Wieters: Skin Dose Determination with the TSEE of BeO thin Film Detectors. Radiat. Prot. Dosim. 17, 97-98 (1986)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
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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.: BI6-A-029-D

Microdosimetry and local dosimetry of ^{226}Ra, ^{239}Pu and ^{241}Am in the beagle dog skeleton.

Head(s) of project: Dr. E. Polig

Prof. Dr. D. M. Taylor

Scientific staff: Dr. E. Polig

Prof. Dr. D. M. Taylor

Frl. A. Stassen

I. Objectives of the project:

To determine the microdistribution of the radiation dose from internally deposited ^{226}Ra, ^{239}Pu and ^{241}Am 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:

a) Continuation of the analysis of ^{226}Ra autoradiographic samples. Conclusion of analysis of lumbar vertebra samples from 10 µCi/kg animals. Begin measurement and analysis of samples from 1 µCi/kg animals.

b) Determination of morphometric parameters and local radiation doses for the beagle skeleton.

c) Dose factors for the osteonal deposition of alpha-emitters in humans and beagle.

III. Progress achieved:

a) Further analysis of lumbar vertebra samples from dogs injected with 10 μCi/kg ^{226}Ra revealed that the difference between formation of new bone and resorption of old bone decreases from an initial value of about 15 /a to 9.6 /a at 1400 days post injection. An investigation of the translocation of hotspots into mineralized tissue shows that the rate of mineralization remains approximately normal (1.13 μ m/d) and also σ_f, the formation interval, is in good agreement with control values. These results indicate that the overall reduction of bone formation is caused by reduced formation areas not by a reduction in the mineralization rate. The augmentation rate of the diffuse label is 0.0058/d (21%/a) and the discrimination factor Ra/Ca for diffusion is 1, i.e. there is practically no discrimination between Ra and Ca.

b) A number of important morphometric parameters can be derived by calculation from input data such as fresh weight, the ratio ash weight/fresh weight, percent cortical bone volume and surface/volume ratio in trabecular and cortical bone. The computational procedure is applicable to both the skeleton as a whole or to parts of it. For the beagle skeleton

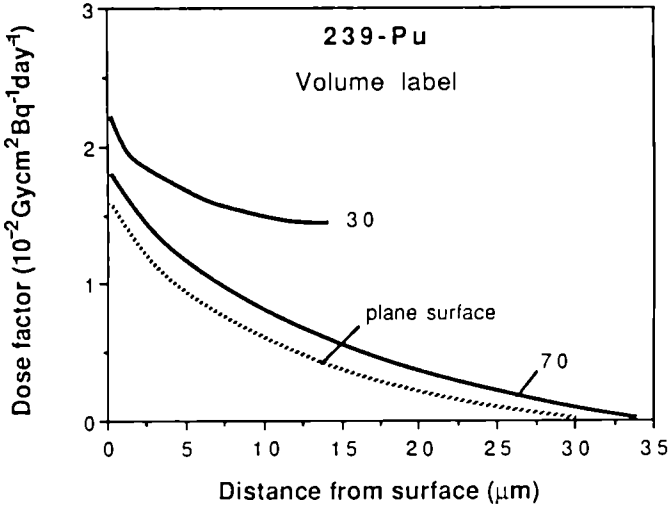

Fig.1 Dose factors for a volume label of ^{239}Pu in human (70 μm dia. of Haversian canal) and beagle osteons (30 μm dia.) Comparison with a plane bone surface. Dose rate = dose factor x concentration.

a total bone mass of 622.3 g and a mass of bone marrow of 339.8 g was obtained. 47.8% of the skeletal volume is bone. The total skeletal surface was determined as 23000 cm^2, of which 68% must be attributed to trabecular bone.

By the additional use of histomorphometric information and data on the retention of alpha-emitters, further parameters such as mean surface concentration, fraction of bone surfaces undergoing formation, dose rates to bone lining cells etc. can be calculated. As an example a calculation for ^{239}Pu in the beagle skeleton has been carried out.

c) To calculate dose factors in osteons of cortical bone, a FORTRAN computer program was designed. The calculational procedure is based on a Monte Carlo simulation of alpha-decays originating in bone which surrounds tissue-filled cylinders (Haversian canals). Dose factors in beagle osteons are significantly larger than in human osteons or at plane surfaces (Fig.1). The effect of "cross-fire" decreases with decreasing diameter of the Haversian canals. At small distances from the wall of the canal, cross-fire contributes about 20-40% of the radiation dose.

An improved expression for the estimation of toxicity ratios was derived. The derivation takes into account different dose factors in trabecular and cortical bone and differing affinities of a radionuclide to these two types of bone. Using this formula, a toxicity ratio $^{239}Pu/^{226}Ra$ of 15.6 results for the beagle and a ratio of 23.0 for humans. The experimentally determined value for the beagle is 16.6.

IV. Objectives for the next reporting period:

1. Continuation of measurement and analysis of autoradiographic samples from beagles with 1 µCi/kg ^{226}Ra. Begin of measurement of samples from 0.3 µCi/kg animals.

2. Begin the analysis of ^{239}Pu autoradiographs (NIAR) from beagle bones.

3. Calculations of hit probabilities to cell nuclei from static and dynamic labels of alpha-emitters in trabecular bone and osteons.

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

University of Utah, Radiobiology Division, Bldg. 351, Salt Lake City, UT 84112, USA.

VI. Publications:

1. Polig E., Jee W.S.S., Dell R., Johnson F.: Local dosimetry of ^{226}Ra. In: Research in Radiobiology, Ann. Report COO-119-262 (1986), p.52.

2. Polig E.: Kinetic analysis of ^{239}Pu retention equations. In: Research in Radiobiology, Ann. Report COO-119-262 (1986), p. 58.

3. Polig E.: Local dose calculations in the skeleton based on morphometric and physiologic parameters. In: Research in Radiobiology, Ann. Report COO-119-262 (1986), p. 67.

4. Polig E., Jee W.S.S.: Bone age and remodeling: A mathematical treatise. Calcif. Tiss. Int. 41, 130 (1987).

5. Polig E., Bruenger F.W., Jee W.S.S.: Quantitative autoradiography of Radium-226 in bone: 1. The measurement technique. Rad. Prot. Dosim. 16, 205 (1986).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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Prof. Dr. R. Jahr
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Bundesallee 100
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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. M. Cosack (until 31.12.1987), Dr. K. Knauf,
Dr. H. Klein (since 1.1.1988)

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:

Additional measurements with the Bonner sphere system will be carried out in monoenergetic neutron fields and in neutron fields with a broad energy range from a number of radio nuclide sources. Reliable response functions for different sets of Bonner spheres should be obtained and the influence of the detector type and size and the density of polyethylene will be studied. An unfolding code will be implemented. A neutron spectrometer based on proportional counters will be connected to a movable personal computer which indicates the spectral flux density on-line. Various materials (e.g. Fe, Al) will be tested in the new calibration facility in order to achieve structured calibration neutron fields of different kind.

III. Progress achieved:

1. Four different Bonner sphere systems are available at PTB, NPL and GSF. The PTB system consists of 14 spheres with diameters from 5.08 cm to 45.72 cm. Before a system can be used for characterising neutron fields it is necessary to determine the responses as a function of energy for each sphere. Two approaches for deriving these response functions are available - calculation and measurement. Both methods are employed in this work because (a) the reliability of the calculation is uncertain, and (b) measurements in monoenergetic neutron fields can only cover a small part of the full energy range. The degree of agreement in the region where both measurements and calculations are possible should give some indication of the reliability of the calculations as a method of interpolating in regions where measurements are impossible.

   In November 1986 during a three week period extensive measurements were performed at PTB (see 1986 Progress Report). In a second period in July 1987 additional measurements were carried out in order to extend the energy range investigated down to 1 keV and up to 14.8 MeV and to reduce the energy gaps in between. Some measurements of the first period were repeated to check the consistency of the results between the two measurement periods.

   The preliminary results were presented at the Sixth Symposium on Neutron Dosimetry at Neuherberg, Munich in October 1987 (see Publ. no. 1). The NPL and PTB sphere systems employing the larger spherical He3 detector are in good agreement throughout the energy range, although the PTB system is consistently 5 % more sensitive. The responses of the PTB system employing a small He3 detector and the GSF system based upon a LiI scintillation counter agree within $\pm$ 10 % throughout the energy range. The results have been compared with two recent calculations published by other groups. Marked discrepancies have been observed between the experimental and the theoretical results both in amplitude and shape which obviously cannot be explained by small differences in the density of the polyethylene material of the spheres.

2. Neutron spectrometry with proton recoil proportional counters: In order to indicate the neutron spectral fluence obtained from the spectrometer by analyzing the measured proton recoil spectrum on-line, a computer (IBM 6150) was installed and the main parts of the required software were developed and put into operation. In looking for calibration procedures with the new calibration facility it was found that passing the neutrons of a Cf252 source through iron will produce a proper calibration spectrum at low energies which also shows lines at 24 keV and at 81 keV. With teflon instead of iron a satisfactory result has not been achieved up to now.

3. Neutron spectrometry with NE213 scintillation detectors:
Three NE213 liquid scintillation detector systems differing in volume by a factor up to 32 were installed and carefully investigated with respect to the proton light output function. For this purpose time-of-flight measurements were performed in a p(20) + Be neutron field with a broad energy distribution. The evaluation of the light output and detector resolution data which are needed for the calculation of the response matrix is in progress.

IV. Objectives for the next 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 spectum for calibration of proportional counters.

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

Dr. J.B. Hunt, Dr. D.J. Thomas, Mr. A.G. Bardell
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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.
   Contribution to the Sixth Symposium on Neutron Dosimetry, Neuherberg, 1987 (to be published in Radiat. Prot. Dosim.)
2. K. Knauf, J. Wittstock
   Neutron Spectrometry with Proton Recoil Proportional Counters at the Research and Measurement Reactor Braunschweig, Status of the Technique.
   PTB-Report, PTB-FMRB-114, Braunschweig (1987)

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:

Intercomparison of prototypes of instruments for practical radiation protection dosimetry based on TEPC's by irradiaton in low-energy filtered neutron beams between thermal and 144 keV. Investigation of tissue-equivalent proportional counters in low-energy neutron fields by using time-of-flight techniques, especially with respect to the influence of the wall thickness and the cavity size on the microdosimetric spectra and the response.

III Progress achieved:

The work for this project has been concentrated on three different subjects:

1. Calibration procedure:
   Since long time the calibration of low-pressure tissue-equivalent proportional counters (TEPC) in units of lineal energy y has mainly been performed by using an internal α-particle source and observing the pulses corresponding to α-particles of nominal energy from the source crossing the detector cavity. The uncertainty of this method, however, is up to 10 %. Now it has been shown that this uncertainty can be strongly reduced, if a complete α-particle spectrum is measured and the upper edge of the spectrum (see Fig. 1) is also used for the calibration procedure (see Publ. No. 3, U.J. Schrewe et al.). This edge corresponds to α-particles which are slowed-down and cross the cavity with maximum energy loss.

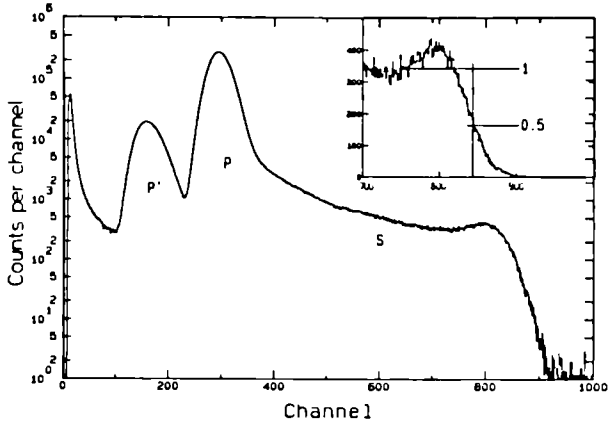


Fig. 1: Pulse height spectrum obtained by a TEPC (cavity diameter: 5.1 cm) with a built-in Cm244 α-particle source.
P events from unscattered α-particles,
P' events from α-particles absorbed by the central wires,
S events from scattered and slowed-down α-particles.

2. Application of time-of-flight techniques:
   Tissue-equivalent proportional counters are used for dosimetry in mixed neutron-photon fields. The discrimination between neutron and photon induced events is usually performed by setting a threshold in the measured pulse-height spectrum or by fitting a pure photon spectrum to the lower part of the pulse height distribution. The accuracy of both methods is limited due to an overlap region from about 1 keV/µm to 10 keV/µm where events from neutrons and photons contribute. In order to study

the different procedures of n-γ discrimination, time-of-flight techniques were applied. With various commercial detector systems a time resolution down to about 80 ns has been achieved. In neutron fields below 5 MeV this resolution is sufficient to study the discrimination techniques and to discriminate between events induced by photons from the target and photons from (n,γ) or (n,n'γ) reactions in the detector.

3. Intercomparison of radiation protection instruments:
Tissue-equivalent proportional counters are able to measure dose equivalent in mixed neutron-photon fields. Several groups in Europe have, therefore, developed prototypes of dose equivalent meters for area monitoring based on a TEPC. In 1986 a first intercomparison initiated by the EURADOS Committee I ("Dose equivalent meters based on microdosimetric technqies") was performed in various reference neutron and photon fields at the PTB. The results have been reported during 1987 (see Publ. No. 2, G. Dietze et al.). In November 1987 a second intercomparison was carried out which included also reference neutron fields at low neutron energies obtained with the filtered beams at the PTB research reactor (thermal, 24 keV and 144 keV neutrons) and at energies of 2.5 MeV and 14.8 MeV produced at the accelerator facility. In 1988 dose equivalent response data for monoenergetic neutrons will be available for all investigated prototypes in the full energy range from thermal up to 14.8 MeV.

IV Objectives for the next 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.

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. H. Schuhmacher, H.G. Menzel, H. Kluge
   Dosimetry of a bare and a D_2O-moderated Cf252 source using low-pressure proportional counters.
   Radiat. Prot. Dosim. 19 (1987) 103 - 109
2. G. Dietze, J. Booz, A.A. Edwards, S. Guldbakke, H. Kluge, J.B. Leroux, L. Lindborg, H.G. Menzel, V.D. Nguyen, Th. Schmitz, H. Schuhmacher
   Intercomparison of dose equivalent meters based on microdosimetric techniques. (presented at 6th Symposium on Neutron Dosimetry and to be published in Radiat. Prot. Dosim.)
3. U.J. Schrewe, H.J. Brede, P. Pihet, H.G. Menzel
   On the calibration of tissue-equivalent proportional counters with built-in α-particle sources. (see No. 2)
4. U.J. Schrewe, H.J. Brede, G. Dietze
   Investigation of tissue-equivalent proportional counters in mixed neutron-photon fields also applying time-of-flight techniques. (see No. 2)

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, R. Hollnagel,
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 spherical and cylindrical phantoms.
Measurement of the free-in-air response and its angular dependence for individual dosemeters to be supplied by CEN, Fontenay-aux-Roses and KFK, Karlsruhe in neutron fields produced at the PTB research reactor.
Measurement and calculation of the response and its angular dependence of the same individual dosemeters on appropriate phantoms.

III. Progress achieved:

The computational study of spherical (ICRU-sphere) and cylindrical phantoms has been completed (see Publ. no. 1, B.W. Bauer et al.). A hollow elliptical cylinder was chosen as a simplified anthropomorphic phantom. The inner void was dimensioned such as to simulate the lung volume. For anterior-posterior irradiation one finds an enhanced and for lateral irradiation a reduced albedo contribution to dose equivalent on this cylindrical phantom as compared to the ICRU-sphere phantom. These effects depend on the cross section density of the phantom material and therefore on neutron energy. An additional computational study revealed a strong influence of the phantom shape on the dose equivalent response of track-etch detectors, especially at higher energies (see Publ. no. 2, R. Hollnagel et al.). Finally, by a computational study on the response of a set of two albedo dosemeters worn on the chest and the back it could be shown that this set allows to reduce the influence of unknown directional distribution of the neutron field quite considerably (see Publ. no. 3, R. Jahr et al.).

The fluence and dose-equivalent responses free-in-air of various individual dosemeters and its angular dependence were measured at all ISO-defined energies between 144 keV and 5 MeV. A new track-etch dosemeter from TASL, Bristol, has been studied at some neutron energies. Furtheron, albedo dosemeters from CEN, Fontenay-aux-Roses and KFZ, Karlsruhe and also a new set of Harshaw dosemeters have been irradiated at PTB and analyzed in cooperation with the other groups. A hollow elliptical cylinder consisting of polytheylen has been built and used in addition to spherical and slab phantoms also consisting of polytheylen. A first intercomparison of calculations and measurements shows quite encouraging results (see Publ. no. 4, W.G. Alberts).

IV. Objectives for the next reporting period:

Computational study of the influence of slab phantoms on detector responses and calibrations 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.

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

G. Portal, CEN, Fontenay-aux-Roses, France and
E. Piesch, KFK, Postfach 3640, Karlsruhe, Germany
D.L. Henshaw, TASL, Bristol, Great Britain

VI. Publications:

1. B.W. Bauer, R. Hollnagel and B.R.L. Siebert
   Numerical Study of the Influence of Phantom Material and Shape on the Calibration of Individual Dosimeters for Neutrons.
   6th Symp. on Neutrondosimetry, München-Neuherberg, 1987
   (to be published in Radiat. Prot. Dosim.)
2. R. Hollnagel, R. Jahr and B.R.L. Siebert
   Influence of Phantoms on the Response of Etched Track Detectors.
   Rad. Prot. Dos. 20 (1987) 25 - 29
3. R. Jahr, R. Hollnagel and B.R.L. Siebert
   Response of a System of Two Albedo Dosemeters worn on Chest and Back in Various Directionally Distributed Neutron Fields.
   6th Symp. on Neutrondosimetry, München-Neuherberg, 1987
   (to be published in Radiat. Prot. Dosim.)
4. W.G. Alberts
   Response of an Albedo Neutron Dosemeter to ^{252}Cf Neutrons on Various Phantoms.
   submitted to Rad. Prot. Dos.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no : BI6-A-024-UK

University of St.Andrews
College Gate
St.Andrews
GB Fife KY16 9AJ

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

Dr. D.E. Watt
Department of Physics
University of St.Andrews
North Haugh
GB St.Andrews KY16 9SS

Telephone number: 334-761.61

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 tests with the prototype counter with laboratory neutron sources and to interpret the results by comparison with the theoretical analyses.

To explore a correction procedure for low energy events due to the fast neutron component.

Upon satisfactory completion of the test to design and manufacture a tissue-equivalent version of the dosimeter.

III. **Progress achieved:** A series of experiments has been performed using the prototype co-axial double cylindrical proportional counter in order to establish its performance. The result of the measurements of the operational charscteristics shows that the counter has acceptable gas gain and resolution as typically applied in microdosimetry. The optimum conditions for operation have been investigated by adding a small amount of 'magic gas' to the filling gas with the object of extending the sensitivity of the counter to lower energy recoils. However, the experiment has so far achieved only limited success. Results for combination of 'magic gas' and TE-gas, in any attempt to increase the counter sensitivity, have not been reported in the literature.

The counter has been fully tested for its response in coincidence and anti-coincidence modes with the outer counter. Input voltages as low as 20mV are easily measurable with this system which minimises the possibility of loss of small pulses generated coincidently in the inner and outer counters. This test has been incorporated with microdosimetric measurements, of a laboratory neutron source, of the response to intermediate energy neutrons. The results have been interpreted quantitatively. They demonstrate that this new type of microdosimeter can be operated to discriminate in favour of intermediate energy neutrons in mixed fields.

Upon satisfactory completion of this preliminary part of the project, an equivalent version of the counter has been constructed except for the TE self-supporting dividing wall of thickness 1.5μm. Special techniques are being developed to prepare uniform thin conducting layers of A-150 plastic.

Theoretical analyses of event spectra of intermediate neutrons for the counter operated with the anti-coincidence arrangement have been calculated to explore the contribution to the response from fast neutrons. Fast neutrons are estimated to contribute a correction factor up to 50% for 1MeV neutrons and 20% for 5MeV neutrons. Empirical techniques are being developed to reduce the errors inherent in application of the correction factor.

IV. Objectives for the next reporting period: To complete laboratory trials with the tissue-equivalent version. To perfect a method for fabrication of the plastic dividing wall. To further study the correction factors required for fast neutron events occuring in the low-energy recoil region and to set-up a computer program for analysis of data prior to formulating it into ROM form.

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: Saion, E B and Watt, D E (1987). Microdosimetry of Intermediate Neutrons in Fast Neutron Fields. Sixth Symposium of Neutron Dosimetry, Munich. 12-16, FRG.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-A-026-UK

European Radiation Dosimetry Group
EURADOS/CENDOS
Radiobiological Institute TNO
P.O.Box 5815
NL-2280 HV Rijswijk

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

Dr. J.A. Dennis
Chairman of EURADOS
NRPB
Chilton, Didcot
GB Oxon OX11 ORQ

Telephone number: 0235/831600/221

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
3. Application of thermoluminescence to routine personal dosimetry.
4. Dissemination and development of computer programs for dosimetric problems. ('Numerical dosimetry')
5. Basic physical data and characteristics of radiation protection instrumentation.

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, A J Waker

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 carry out the second part of the intercomparison of microdosimetric instruments for area monitoring with neutrons including measurements with low energy neutrons in filtered reactor beams. To prepare a final report on the results of the first part of the intercomparison.
To continue benchmark calculations with different computer codes for the compilation of microdosimetric spectra produced by neutron radiations and to continue the development of programs for the calculation of microdosimetric spectra from photon radiations.

III Progress achieved:

The results of the first part of the intercomparison of radiation protection instruments based on microdosimetric principles at Physikalisch-Technische Bundesanstalt have been reported in a PTB Report (PTB-ND-29 (1986), preliminary results) and at the 6th Symposium on Neutron Dosimetry at Neuherberg in October, 1987. The final report is completed and is being prepared for publication by the CEC. This report on the intercomparison of five different microdosimetric dose equivalent meters in the fields of monoenergetic neutron sources (0.073-5 MeV), a D_2O moderated ^{252}Cf source and a ^{60}Co source provides results in terms of dose equivalent, absorbed dose, quality factors, neutron and gamma ray dose fraction and comparison of lineal energy spectra (for part of the participating systems). There is also a detailed analysis given of statistical uncertainties which is a more complex problem for tissue-equivalent proportional counter (TEPC) measurements than most conventional dose meters because dose equivalent is not only determined by the pulse frequency but also by the pulse heights. As expected, the detector sensitivity was shown to depend on detector size and radiation quality. There were, however, additional influences established as, for example, the detector wall thickness at low neutron energies. The influence of calibration, detector design, shape and size of the sensitive volume and pressure of the gas filling on the results are discussed in the report.

Although final conclusions on the performance of TEPC instruments and recommendations will have to include the results of the second part of the intercomparison (see below) relevant findings have been established in this first intercomparison: (1) The calibration procedure has to take account of the phantom based definition of the quantity ambient dose equivalent, H*(10). (2) The parameters with significant influence on the response of TEPC systems are wall-thickness, simulated diameter and the procedure to evaluate the quality factor. These parameters can therefore be used to further improve the response.

In November/December 1987 the second part of the intercomparison was carried out, again jointly organised by PTB and Committee 1 of EURADOS-CENDOS. Quasi-Monoenergetic neutrons (thermal neutrons, 24.5 and 144 KeV neutrons) were provided by the reactor facility FRMB of PTB (filtered reactor beams). At the accelerator facility monoenergetic neutrons of 0.57, 2.5 and 14.8 MeV were available. In addition, measurements were performed at a ^{60}Co and a D_2O moderated ^{252}Cf-source. Two additional institutions (EIR, Würenlingen (CH) and University of Leeds) joined the group of participants of the first intercomparison (CEN de Fontenay-aux-Roses, CEN de Grenoble, KFA Jülich, NIRP Stockholm, University of Saarland). A conventional neutron dose equivalent meter (Rem counter) was operated by PTB in the intercomparison. At present results are being collected.

Reports on the second intercomparison and summary of both parts will be presented at the second Workshop on Implementation of Dose Equivalent Meters Based on Microdosimetric Techniques in Radiation Protection to be held at Schloss Elmau (FRG) from October 18 to 20, 1988. This workshop will be jointly organised by the Commission of the European Communities and the GSF, Neuherberg together with EURADOS.

The objectives for the reporting period have been fully achieved with regard to the intercomparison programme. The assessment of the progress of the other activities is more difficult. The calculations of the response of TEPC to photons, in particular low energy photons are very complex so that results are not anticipated before 1989. The development of a reference code for energy deposition calculations for neutron irradiations and benchmark calculations are being carried out.
Progress, however, has been slower than anticipated due to technical and organisational reasons. The necessity to link neutron transport calculations with energy deposition codes has become very urgent in view of the results of the intercomparison. With regard to this problem a combined effort of EURADOS project groups 1 and 4 is expected.

IV Objectives for the next 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.

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

CEN, Fontenay-aux-Roses
CEN, Grenoble
DKFZ, Heidleberg
GSF, Neuherberg
KFA Jülich
NIRP, Stockholm
NRPB, Chilton
PTB Braunschweig
University of Leeds
University of Saarland, 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. Presented at 6th Symposium on Neutron Dosimetry, Oct. 1987, Munich - Neuherberg and to be published in Radiat. Prot. Dosim.

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 a EURADOS-Intercomparison, prepared for publication by the CEC.

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 continue the preparation of a review of survey instruments for skin dosimetry and surface contamination monitoring. To perform calculations in the field of low-energy photon dosimetry aiming at an improved accuracy. To prepare a concluding report on the importance of problems of skin dosimetry and surface contamination monitoring. To continue preparations for a benchmark intercomparison of computational methods for beta radiation dosimetry. To make measurements and calculations of dose rates from particulate beta-ray sources. To intercompare dose-rate measurements of equal ^{147}Pm-sources using different measurement methods. To continue the co-operation with the Directorate General V in the organisation and data evaluation of an intercomparison of personal dosemeters for beta radiation dosimetry. To continue the evaluation of the biological effectiveness of low-penetrating radiations in co-operation with EULEP.

III. Progress achieved:

The preparation of a review document on dose rate meters for skin dose measurements has continued and the final document is expected to be ready in 1988. The preparation of a document dealing with instruments for monitoring of surface contamination is planned to start in 1988. The accuracy of transport calculations concerned with low energy photon radiation has been improved by introducing more precise values of the probabilities of the interaction processes. Calculations have been performed for a 80 keV point source located at the centre of an ICRU sphere. The calculations were made by a computer program developed at CPA, Toulouse. Plans have been made also to use computer programs applied at KFA, Jülich and PTB, Braunschweig. Detailed programs 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 have been set up. The measurements of dose rates from the $^{90}Sr/^{90}Y$ source will take place at PTB, Braunschweig and those of the ^{60}Co sources at CEGB, Berkeley and CEN, Grenoble. The calculations will be performed at CPA, Toulouse. The experimental work will take place during 1988. Further evaluations have been made of the responses to a questionnaire concerned with problems of skin dosimetry at the working place and a report of the results was presented at the CEC Beta Intercomparison Information Seminar held at Bologna, 25-27 May 1987. The results will be published in the proceedings of this seminar. A project with the purpose of preparing experimental data on skin dose rates from typical beta ray sources from the working place has been initiated. In this connection four, carefully prepared, equal, 4 cm x 4 cm, ^{147}Pm sources, mounted on equal holders have been prepared and distributed to four laboratories (NRPB, PTB, Fontenay-aux-Roses, and Risö) for making comparative measurements of dose rates at different distances and angles from the sources. The measurements will take place during 1988. Members of the Committee have contributed with advising the Directorate General V on the organisation and data evaluation of an intercomparison of personal dosemeters for beta dosimetry. Reports on this work will be published in the proceedings from the CEC Beta Intercomparison Information Seminar held at Bologna, 25-27 May 1987. The evaluation of data on the biological effectiveness of low-penetrating radiation has continued in co-operation with EULEP, who are now taking part in the work of a Task Group set up by ICRP to deal with these problems.

IV. Objectives for the next 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 atudy 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.

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

RNL, Risø
NRPB, Chilton
PTB, Braunschweig
SPEE/LMR, Grenoble
GSF, Neuherberg
CEGB, Berkeley
CEN, Grenoble
RSO TNO, Arnhem
KFA, Jülich
KFZ, Karlsruhe
NPL, Teddington
CPA, Toulouse

VI. Publications:

Christensen, P, Herbaut, Y, and Marshall, T O.
Personal Monitoring for External Sources of Beta and Low Energy Photon Radiations. Radiat. Prot. Dosim. 28, 241-260 (1987).

Patau, J P, Casanovas, A M, and Oustrin, J.
Accuracy of Transport Calculations Concerning Low Energy Photon Radiations. (October 1987). In press.

Title of the project no.:

3. Application of Thermoluminescence to Routine Personal Dosimetry

Head(s) of project:

J R Harvey

Scientific staff:

J R Barthe, G Busuoli, P Christensen, K E Duftschmid, H W Julius, J Böhm, M Marshall, T O Marshall, M Oberhofer, D Regulla

I. Objectives of the project:

Study and resolution of problems encountered in the application of thermoluminescence dosimetry techniques in individual and environmental monitoring

II. Objectives for the reporting period:

The two unpublished reports will be published in the open literature. Reprints of all six reports listed above will be bound in a single volume and distributed to associate members of EURADOS-CENDOS.

III. Progress achieved:

The year was spent collating the reports produced during the working life of this committee. Two papers have been published, one on the relative tissue kerma sensitivity of thermoluminescent materials to neutrons and the other a report of a thermoluminescence dosimetry workshop held in Ispra, Italy in 1985. Reprints of all the reports produced by the committee were bound into a single volume which was distributed to all members of EURADOS-CENDOS.

IV. Objectives for the next reporting period:

The tasks undertaken by this committee have now been completed and the committee has been disbanded.

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

CEGB, Berkeley
CEA, Fontenay-aux-Roses
ENEA, Bologna
RNL, Riso
OFZS, Seibersdorf
RSO TNO, Arnhem
PTB, Braunschweig
AERE, Harwell
NRPB, Chilton
CEC JRC, Ispra
GSF, Neuherberg

VI. Publications:

J A B Gibson, The relative tissue kerma sensitivity of thermoluminescent materials to neutrons. Radiation Protection Dosimetry Vol. 15, No.4, pp.253-266

J R Barthe, J Bohm, P Christensen, C M Driscoll, J R Harvey, H W Julius, M Marshall, T O Marshall and M Oberhofer, Report on a workshop on the Application of Thermoluminescence Dosimetry to Large Scale Individual Monitoring. Ispra, 11-13 September 1985. Radiation Protection Dosimetry, Vol. 18, No. 1, pp.47-61.

EURADOS-CENDOS, Aspects of Individual Monitoring. Ed. J R Harvey. Published by Nuclear Technology Publishing, Ashford, Kent, UK, 1987.

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

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. Final Report on the californium-252 benchmark problems.
2. Benchmark studies on the exposure of a phantom by extended area sources (homogeneously distributed caesium-135). Intercomparison of H_{eff} derived from computed organ doses. Selection of anthropomorphic and simplified phantoms for further studies.
3. Benchmark calculations for Bonner Spheres.
4. Intercomparison of unfolding codes for Bonner spheres.
5. Improvement of database and code for charged particle calculations. Supporting calculations for EURADOS Committee 1 (especially for the intercomparison of tissue equivalent proportional counter measurements in 2 keV filtered reactor beams; NBS + PTB).

III Progress achieved:

The intercomparison of calculations of spectra from ^{252}Cf sources moderated by D_2O and H_2O spherical moderators of various sizes has been completed and will be published. The main conclusion is that moderated ^{252}Cf sources can serve as well described and relatively inexpensive neutron sources. However, the particulars of each facility (eg, room size, construction of set up and source containment) need to be taken account of by appropriate calculations. The final agreement of the calculations (delivered by colleagues from AERE Harwell, GSF, Munich, IKE, Stuttgart and PTB, Braunschweig) is quite satisfactory. An important by-product of this intercomparison is an improved set of input data for this kind of calculations.

An intercomparison of computer codes as applied to multisphere (ie, Bonner sphere) spectroscopy and dosimetry in neutron fields has been initiated. In a first step numerically simulated Bonner sphere readings are used as input. This serves to examine the unfolding capability of the different codes without perturbations due to experimental variances and covariances. In a second step the readings were statistically altered in order to simulate experimental variances.

A preliminary evaluation of five contributions so far received allows to recommend the multisphere system as a useful instrument for practical neutron spectrometry and dosimetry in radiation protection environments.

The quality of unfolded spectra and derived dose equivalent depends on the use of correct response functions. Therefore, the committee initiated an intercomparison of calculations. In a first contribution based on the ANISIN code it was found that the natural differences in the density of polyethylene moderators led to strong variations in the response functions.

No progress has been made in the selection of appropriate anthropomorphic phantoms. It is understood that ICRP is preparing a report on this subject.

There has been slight progress in establishing a definitive computer code for the computation of charged particle spectra resulting from neutron irradiations. No progress has been made in the establishment of a similar code for photon radiations.

IV. Objectives for the next 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.

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

AERE, Harwell
CEA, Fontenay-aux-roses
CEN, Cadarache
GSF, Munich
IKE, Stuttgart
NPL, Teddington
NRPB, Chilton
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 Dietz, S Guldehakke, V D Haigh, V E Lewis, D R Schlegel-Bickman, H Schraube, U J Schrewe
2. Track-Etch Detectors: D T Bartlett, J-L Decossas, K G Harrison, J R Harvey, L Lembo, R Medioni, E Piesch, H Schraube, L Tommasino, J-C Varlelle, 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:

Occupational exposures will be inferred from systematic enquiries, working place analysis and field studies. A specific example of such a type of study is the determination of neutron spectra in PWR containments to evaluate appropriate quality factors.

The activities of the working group on the application of track detectors in neutron dosimetry will be continued. A European workshop on the application of track detectors is to be held in May 1987.

III. Progress achieved:

The use of Mg/Ar ionisation chambers for determination of the photon absorbed dose in mixed neutron-gamma-ray fields has been shown to have specific advantages. Compared to C/CO_2 ionisation chambers, Mg/Ar chambers have a considerably smaller relative neutron sensitivity (k_u). The k_u-values of Geiger-Muller (GM) counters are smaller than those of Mg/Ar ionisation chambers, however, the energy dependence of the response of Mg/Ar ionisation chambers to photons is considerably smaller than that of (energy compensated) GM counters.

In a previous joint study by PTB-Braunschweig, FRG; GSF-Neuherberg, FRG and TNO-Rijswijk, Netherlands, performed within the framework of CENDOS, the characteristics of Mg/Ar ionisation chambers were investigated (Zoetelief et al, Phys. Med. Biol. 31, 1339, 1986). Among the characteristics studied were the dependence of the reading on gas flow rate, chamber wall thickness and charge collection, and the relative neutron sensitivity. A major conclusion is that the relative neutron sensitivity for different chambers of the same design (Exradin-MG2) in the same neutron beam show considerable variations.

A workshop on 'The Development of Personal Neutron Dosemeters Based on Tract Etch Detectors' was organised at AERE, Harwell, May 12-14, 1987. The workshop was co-sponsored by the UKAEA Harwell Laboratory, EURADOS-CENDOS, and the Commission of the European Communities, Directorate General for Science, Research and Development, Radiation Protection Programme.

The workshop was attended by approximately 45 scientists representing most of the groups in Europe and continental America actively engaged in track-etch neutron dosimetry. Invited papers were given on: characteristics of neutron fields, quantities and units, fundamentals of neutron response, ageing and environmental effects, polymer physics, activities in the USA, and future developments. Contributed papers were given on many different neutron dosimetry systems based on track-etch. The workshop concluded with a round-table discussion. The delegates expressed the view that the workshop had been extremely valuable. The proceedings will be published by Nuclear Technology Publishing.

A joint European/USA/Canadian joint irradiation of track-etch detectors was undertaken in the Autumn of 1986. Irradiations of the detectors, which were sent by post, were undertaken by: AERE Harwell; PNL, Richland; PTB, Braunschweig; GSF, Neuherberg; and CRNL, Chalk River. Neutrons were provided at 144 keV, 570 keV, 1.2 MeV, 5.3 MeV, 14.7 MeV, and from Cf-252 sources. Groups from the following 13 laboratories provided track-etch detectors: KfK, Karlsruhe; ENN-DISP, Rome, NRPB, UK; ENEA, Bologna; CRNL, Chalk River; LEPOFI, Limoges; AERE, Harwell, LLNL, Livermore; CEGB, Berkeley; RNL, Riso; CEN, Fontenay-aux-Roses; PNL, Richland and Bristol University Physics Department.

The results of the joint irradiation were compiled and published, together with introductory remarks and conclusions during the course of 1987. (Neutron Irradiations of Proton-sensitive Track Etch Detectors: Results of the Joint European/USA/Canadian Irradiations.

E Piesch. KfK 43054 EURADOS/CENDOS Report 1987-01, September 1987).

A committee meeting was held on 19 October 1987 at GSF Munich following the Sixth Symposium on Neutron Dosimetry. The results of the recent joint irradiation and the future programme were discussed. It was concluded that the 1986 joint irradiation had been very useful and there was widespread support for another, possibly in 1988. A detailed proposal was prepared. It was felt that as well as being a useful and economical source of well calibrated neutrons, such joint irradiations gave delegates an insight into the performance of their systems vis-a-vis those developed at other laboratories. It was also felt that the background characteristics of the etch plastics should be considered in the future programme. These characteristics, which are currently causing some problems, will become more important if quality factors rise or permitted radiation levels are reduced.

IV. Objectives for the next 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.

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
CEA, Fontenay-aux-Roses
CEGB, Berkeley
ENEA, Bologna
ENEA, Rome
KfK, Karlsruhe
Limoges University
NRPB, Chilton

VI. Publications:

E Piesch, Neutron Irradiations of Proton-Sensitive Track Etch Detectors: Results of the Joint European/USA/Canadian Irradiations. KfK 4305 EURADOS-CENDOS Report 1987-01, September 1987.

Neutron Irradiations of Proton Sensitive Track Detectors: Results of a Joint Irradiation Organised by CENDOS. Ed. K G Harrison, August 1985, AERE Harwell Laboratory Report, AERE-R11926.

Title of the project no.:

6. Assessment of Internal Dose

Head(s) of project:

J A B Gibson

Scientific staff:

P J Darley, Miss F A Fry, K Henrichs, R Kunkel, J Piechowski, R Roth, H Schiefendecker, R G Thomas (corresponding member)

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 establish 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.

III. Progress achieved:

There was one meeting of the group in October 1987 where it was agreed that a programme of work be established under 7 headings.

(i) Models; excretion (long and short-term), metabolic uncertainties, effects of health, chemicals, etc;

(ii) air sampling; PAS v SAS and use of alarm air samplers, statistical interpretation, effect of transportability (D,W or Y);

(iii) in-vivo monitoring; interference from Chernobyl, partial body monitoring;

(iv) bioassay; urine data within first 24 h, faecal sampling, frequency and interpretation;

(v) autopsy data; availability and information exchange;

(vi) dose records; compatibility;

(vii) information exchange.

The following specifications were identified.

(a) List elements of interest in RP (Miss F A Fry by 1.11.87).

(b) Proposals for stable element metabolic studies (P Roth by 1.12.87, Abstract, and 31.3.88).

(c) Models for elements identified in (a) (K Henrichs by 31.3.88).

(d) Identification of source of autopsy data in FDR (K Henrichs), France (J Piechowski), USA and UK (Miss F A Fry and J A B Gibson - all by 30.11.87).

(e) Dose record format; FDR (KH), France (JP), UK (FAF), OECD (J A Dennis).

(f) Database - metabolism and models (JABG and Miss FAF by 1.12.87 abstract and 31.3.88).

(g) Laboratories assessing internal dose; FDR (H Schiefendecker (KH) by 30.11.87), France JP by 30.11.87) and Italy (J A Dennis).

IV Objectives for the next reporting period:

To carry out the work specified in Section III above. The group would meet informally at the CEC Workshop on Biological Assessment of Occupational Exposure to Actinides, 30.5.88 to 2.6.88 in Versailles. The next main meeting would be in Frankfurt on 18-20 October of 1988. The inclusion of a parallel session on Assessment of Internal Dose at the SRP International Symposium, Malvern, 4-9 June 1989 would be discussed with the organisers.

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

UKAEA, Harwell
CEGB, Berkeley
NRPB, Chilton
University of Saarland, Homburg
CEA/ISPN, Fontenay-aux-Roses
GSF, Frankfurt
GSF, Neuherberg
KFK, Karlsruhe
US DOE, Washington

VI. Publications:

No publications in this period.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

International Commission on
Radiation Units and Measurements
ICRU
7910 Woodmont Avenue, Suite 1016
USA Bethesda, MD 20814

Contract no.: B16-A-027-USA

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) the use of computers in external beam radiotherapy procedures with high energy photons and electrons, (2) tissue substitutes in radiation dosimetry and measurements, (3) determination of dose equivalents for external radiation sources--part 2. Completion of drafting work (consideration by the ICRU) of reports on: (1) clinical neutron dosimetry (physics aspects), (2) definitions of physical parameters to specify performance of imaging instruments in nuclear medicine and (3) determination of absorbed dose distribution around a source used for interstitial therapy

III. Progress achieved:

During 1987 the International Commission on Radiation Units and Measurements (ICRU) completed work on three reports which are now either in press or at the stage of preparing the printer's manuscript:

(1) ICRU Report 42, Use of Computers in External Beam Radiotherapy Procedures with High-Energy Photons and Electrons (in press)

(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 (preparing printer's manuscript)

ICRU Report 42, Use of Computers in External Beam Radiotherapy Procedures with High-Energy Photons and Electrons, continues the series of reports (ICRU Reports 23, 24, 29) dealing with dosimetric problems in external beam-therapy with high-energy photons. Since the publication of these reports, computers have played an increasingly important role in dosimetric and treatment-planning procedures in an increasing number of therapy centers. The report should prove valuable to therapists and physicists who use computers as an integral part of treatment planning and will help call attention to the many details associated with treatment planning.

ICRU Report 43, Determination of Dose Equivalents from External Radiation Sources--Part 2, is, as the title indicates, the second in what will be a three part series on determination of dose equivalents. ICRU Report 39, defined new quantities for use in the monitoring of individual exposures and areas where individuals can be exposed. ICRU Report 43 now follows this up with an exposition of the basis for the defined quantities and information on how the dose equivalents received by exposed individuals may be determined when additional data on irradiation conditions are available.

ICRU Report 44, Tissue Substitutes in Radiation Dosimetry and Measurements, provides information on the need for carefully selecting materials for use in constructing phantoms and radiation detectors which are necessary for the measurement of absorbed doses within and around

irradiated body tissues. For a given radiation energy and type, the materials should absorb and scatter the radiation to the same extent, within known acceptable limits, as the irradiated tissue. This report should lead to improved accuracy in the determination of absorbed dose in radiotherapy, radiodiagnosis, radiation protection and radiobiology.

ICRU's review of a draft report on the physics aspects of clinical neutron dosimetry was completed during 1987 and modifications based on the results of the review are now being made. As soon as this is completed preparation of the printer's manuscript can begin.

Sufficient progress was made during 1987 on the drafting of a report on physical parameters to specify performance of imaging instruments in nuclear medicine that it is expected that this report will be reviewed by the ICRU in 1988.

Work continued during 1987 on the development of reports concerned with (1) absorbed dose standards for photon irradiation and their dissemination, (2) chemical dosimetry, (3) clinical dosimetry for neutrons--dose specification for reporting interstitial therapy, (5) measurement of dose equivalent, (6) phantoms for therapy, diagnosis and protection, (7) statistical methods used in particle counting, and (8) stopping power for protons and alpha particles.

Recently initiated are efforts concerned with (1) imaging, (2) revision of recommendations on dose specification for reporting external beam therapy with photons and electrons, and (3) stopping power for heavy ions.

The ICRU is studying the need for new work concerned with (1) concepts, quantities and units, and measurement techniques for characterization of materials-effects studies, (2) hyperthermia and (3) magnetic fields.

IV. Objectives for the next 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) the basis for quantities used in determination of dose equivalent from external radiation sources, (3) tissue substitutes in radiation dosimetry and measurements and (4) physics aspects of clinical neutron dosimetry

Completion of the drafting work on a report on physical parameters to specify the performance of imaging instruments in nuclear medicine.

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 (in press)

ICRU Report 43, Determination of Dose Equivalents from External Radiation Sources--Part 2 (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
Bureau International des Poids
et Mesures
Pavillon de Breteuil
F-92310 Sèvres

Telephone number: 14-271-2414-755

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:

Initiation of the drafting work on an ICRU report on measurement of dose equivalent.

III. Progress achieved:

During 1987 the ICRU report committee responsible for the drafting work on an ICRU report on measurement of dose equivalent began its work. An outline for the report was developed which includes the following topics:

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)

Drafting work was initiated and drafts of some sections of the report were reviewed by the report committee and modifications agreed upon.

IV. Objectives for the next reporting period:

Completion of the drafting work and revision on the basis of the comments of the report committee.

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

1987

Contractor: Contract no.: BI6-B-030-DK

Risø National Laboratory
DK-4000 Roskilde

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 foodchain and b) determination of less wellknown long-lived radionuclides.

Head(s) of project:

Dr. Sven Poul Nielsen(a), Dr. Elis Holm(b)

Scientific staff:

Lars Bøtter-Jensen(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 wellknown long-lived radionuclides in low concentrations in environmental samples.

II. Objectives for the reporting period:

a) Suitable data sets from the Chernobyl contamination of the human foodchain will be identified and used for model calculation, as well as fallout data from other Nordic countries. Further model development and comparisons will be made in the context of BIOMOVS.

b) β-spectroscopy using passivated implanted planar silicon detectors will be applied in order to check Tc-counting samples for imparities. The radiochemical procedure for Tc will be improved in order to remove Ru from the samples. The development and application of windowless β-counters will be continued.

III. Progress achieved:

a) Dynamic models

The ^{131}I data collected in Denmark, Finland, and Sweden after the Chernobyl accident have been used for a comparison between predictions from fallout models and actual observations. Data on airconcentrations (particulate and gas), total deposition (soil samples), grass and milk were used.

In order to eliminate daily variations time integrated data were applied.

It appeared that the model based upon the observed air concentrations of ^{131}I overestimated the deposition by a factor of 2, the grass levels by a factor of 10 and the milk concentrations by a factor of 100. The variation between the 3 countries were least for the deposition and grass levels (relative geometric SD a factor of 1.2) and highest for milk (relative geometric SD a factor of 2.4). The reason for the great discrepancies for milk was primary due to the fact that only very few cows in the Nordic countries were grazing when the Chernobyl fallout arrived. The reason for the other overstimates by the model were 1) the Chernobyl fallout came in the very beginning of the growing season, when the growth dilution and field loss are high and when the initial retention on the crops is low. 2) the rate of deposition of the fallout was high, as it came in showers, this also reduced initial deposition and enhanced field loss. 3) the physicochemical properties of the Chernobyl debris may have been different from those of global fallout.

The Cherobyl data are being compiled in a Nordic data base, which is compatible with the CEC REM-bank in Ispra.

b) Determination of less wellknown long-lived radionuclides

Tc-99 radiochemical analysis

After the Chernobyl accident it was noticed that the ^{99}Tc counting samples contained impurities of ^{103}Ru, ^{106}Ru, and ^{110m}Ag. The analytical method used did apparently not decontaminate efficiently for these radionuclides. The main effort in 1987 within this project has therefore been to develope a new method for ^{99}Tc analysis. This task has been very pertinent because it was necessary to ensure that the Chernobyl accident did not pertubate the marine radiotracer studies, where ^{99}Tc probably now is the only usuable tracer after the Chernobyl accident.

A method for analyzing ^{99}Tc in environmental samples has been developed applying solvent extraction in which the valences of Tc and Ru are controlled with H_2O_2 and NaOCl. Tc and Ru, which are oxidized to TcO_4, and RuO_4 by NaClO are separated by extraction with CCl_4 at P_H=4. The RuO_4 is reduced to low valence and technetium is kept in the TcO_4 state with H_2O_2. Tc, Ru, and other nuclides are subsequently separated by solvent extraction with cyclohexanone and 5% TIOA/xylene. The decontamination of the procedure is 1.35×10^5 for ^{103}Ru and $1.66 \cdot 10^5$ for ^{110m}Ag. The chemical yield of ^{99}Tc is 55%.

A procedure for purification of ^{99}Tc from electrodeposition plates was also developed. This procedure was successfully applied to a number of analyses carried out according to the old ^{99}Tc analysis (cf. project 2b: Field studies).

Development of low-level beta counters based on Passivated Implanted Planar Silicon detectors

A desire to use beta spectrometry in connection with low-level beta counting led to the development of a prototype anticoincidence beta counter based on Passivated Implanted Planar Silicon (PIPS) detectors. The research work was based on many years of experience with the development of gas flow anticoincidence GM counters for ultra low-level beta counting applications.

Two types of PIPS detectors with an active area of 450 mm^2 were investigated. One was a partially depleted type with a depletion depth of 100 microns and another was a fully depleted type with a depletion depth of 500 microns. The two PIPS detectors were arranged in a mechanical set-up with a common square formed guard counter placed on top of the sample counters. An optimal guard effect was obtained by using PIPS detectors mounted with radial microdot connector to allow the detectors to be placed in a close geometry to the guard counter. Initial measurements were carried out with the gas flow guard counter operated in GM mode.

The PIPS detectors were coupled to a Canberra preamplifier type 2003BT and to a home designed electronic module incorporating linear amplifier and anticoincidence gates. In total count mode a background in a 100 mm lead shelding of approx. 0.5 cpm was obtained. The efficiency of a ^{136}Cl test source (activity deposited on 25 mm diameter plastic disc) was measured to be approx. 44%.

The spectrometry properties of the beta counter - when using anticoincidence technique - is being investigated.

IV. Objectives for the next 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 ionexchange 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.

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. G.M. Smith)

VI. Publications:

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.

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:

a) Samples from the 85-86 excretion experiment will be analysed and reported. A full salinity follow-up study is planned. Furthermore, the experiments on the mechanisms of Tc accumulation and retainment in Fucus will be continued.

b) In June 1987 we will join a cruise to the Greenland Sea on board the F/S Polarstern. We will continue the studies of the Chernobyl debris in the Baltic Sea and the Danish Straits. The compartment model developed for the NE Atlantic will be validated using ^{99}Tc and if possible Chernobyl debris.

c) Studies of other elements than Pu and Am, e.g. U and Th will be carried out in the Thule sediment and the sedimentation rate studies will be reported.

III. Progress achieved:

a) Experimental studies

A new one year loss experiment with Mytilus was started in the Mediterranean in collaboration with the IAEA laboratory in Monaco during November/December 1987. Several mussels were contaminated with ^{60}Co, ^{65}Zn, ^{106}Ru, ^{110m}Ag, ^{134}Cs, ^{154}Eu, ^{239}Pu, ^{241}Am, and ^{244}Cm before they were put out in the Mediterranean in small net bags. Samples will be recovered monthly during 1988. This experiment is to be comapred with earlier experiments performed with the same methodology in the Baltic under very different environmental conditions.

During 1987 most samples from the earlier long-term loss experiment with Mytilus and Fucus in the Baltic were analysed. Preliminary data analyses indicate differences in the behaviour of plutonium added to the experiment in different oxidation states (+IV va. +VI). The reduced form is accumulated faster than the oxidised form in both mussels and seaweed. This can be exolained by the higher affinity for particles (filtered by the mussels) and other surfaces (e.g. the surface of seaweeds) for reduced plutonium. However, plutonium taken up from the reduced state is initially also lost much faster from mussels than oxidised plutonium, whereas the loss rates after two weeks are identical. Probably this shows that the loss of the two different isotopes after two weeks is from the same state. For fucus vesiculosis the faster loss of the plutonium isotope added in the reduced state appears to continue for at least some months. This could indicate that the reduced plutonium stays closer to the surface of the plants, which is gradually lost, whereas the plutonium isotope initially present in the oxidised state might penetrate deeper into the tissues.

b) Field studies (North Atlantic region - Baltic Sea)

The group participated in the F/S Polarstern cruise in June 1987 to the Greenland Sea. Approximately 100 seawater samples were collected for radiocesium (54), ^{90}Sr (37), ^{99}Tc (25), Pu (18), and Am (18) analysis. The ^{99}Tc were precipitated from large volumes (0.2-1m^3) of water with ^{99m}Tc added on board as yield determinant. The sampling this year shall

be seen in context with the socalled Greenland Sea Project (GSP), which is a cooperation between scientific institutes from 8 countries aimining at a better understanding of the processes going on in this part of the NE Atlantic. The sampling in June was supplemented by another one in September taking place in the southern part of theGreenland Sea. Approximately 20 samples were here collected from the Islandic ship Bjarni Sämundsson. Finally, the Greenland Fisheries and Environmental Research Institute collected 14 seawater samples along the West coast of Greenland in Aug.-Sept. 1987.

The results of the ^{99}Tc analysis from the F/S Gauss cruise to the Baltic Sea in Oct.-Nov. 1986 show that the Chernobyl accident did not contribute significantly to the ^{99}Tc levels in the marine environment. Hence one may still use ^{99}Tc as an oceanic tracer in the NE-Atlantic.

c) Thule studies

The analysis of the sediment samples for ^{210}Po and ^{210}Pb from the 1984 sampling has been continued by our Canadian colleagues at Bedford. No results are however yet available. The results from the 1984 expedition have been published.

IV. Objectives for the next reporting period:

Samples from the Mediterranean loss experiment will be taken during 1988, and further laboratory experiments on effet 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.

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

N.S. Fisher, Brookhaven Nat. Lab., USA

Manuella Notter, SNV, Sweden

P. Guequeniat, CEA, France

A.D. Bettencourt, L.N.E.T.I., Portugal

Elis Holm, 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

VI. Publications:

Lars Hallstadius, Estrella Garcia-Montano, Ulf Nilsson, and Søren Boelskifte. An Improved and Validatel Dispersion Model for the North Sea and Adjacent Waters. Journal of Environmental Radioactivity 261-274 (1987).

A. Aarkrog, S. Boelskifte, H. Dahlgaard, S. Duniec, L. Hallstadius, E. Holm, and J.N. Smith. Technetium-99 and Cesium-134 as Long Distance Tracers in Arctic Waters. Estuarine, Coastal and Shelf Science 637-647 (1987).

A. Aarkrog, S. Boelskifte, H. Dahlgaard, S. Duniec, E. Holm, and J.N. Smith. Studies of Transuranics in an Arctic Environment. Journal of Radioanalytical and Nuclear Chemistry, Articles 39-50 (1987).

A. Aarkrog, S. Boelskifte, L. Bøtter-Jensen, H. Dahlgaard, Heinz Hansen, and S.P. Nielsen. Environmental Radioactivity in Denmark in 1985. Risø-R-540 (1987).

A. Aarkrog, S. Boelskifte, E. Buch, G.C. Christensen, H. Dahlgaard, L. Hallstadius, H. Hansen, E. Holm, and J. Rioseco. Environmental Radioactivity in the North Atlantic Region, The Faroe Islands, and Greenland included, 1985. Risø-R-541 (1987).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no : BI6-B-183-D

Technische Hochschule Darmstadt
Karolinenplatz 5
D-6100 Darmstadt

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

Prof. Dr. K. Bächmann
Fachb.f.Anorgan.Chemie u.Kernchemie
Technische Hochschule Darmstadt
Hochschulstrasse 4
D-6100 Darmstadt

Telephone number: 06151/161

Title of the research contract:

Investigation of the lead 210 pathways via waste air and waste water of uranium mining sites.

List of projects:

1. Investigation of the lead 210 pathways via waste air and waste water of uranium mining sites.

Title of the project no.:

No.BI-6-0183-D (B): "Untersuchung der Transportwege von ^{210}Pb über Abluft und Abwasser von Uranminen"

Head(s) of project:

Prof. Dr. K. Bächmann

Scientific staff:

H. Klenk

Dr. Ing. G. Hartmann

Ing. E. Lisson

Dipl. Ing. D. Kubin

I. Objectives of the project:

The general mechanisms of distribution of ^{210}Pb from areas with increased natural radioactivity with special reference to formation, transportation and environmental deposition of ^{210}Pb is investigated. To determine the air pathways of ^{210}Pb, air filter and cascade impactor samples are collected; to estimate its environmental distribution, soil samples should be included. For evaluation of these samples, total deposition, inactive elements (e.g. Fe, Mn, Al, Zn), ^{210}Pb, ^{226}Ra, U_{nat} and their correlations should be determined.

II. Objectives for the reporting period:

1. Determination of the environmental distribution of ^{210}Pb by analysis of soil samples (^{210}Pb, ^{226}Ra, inactive elements) at suitably chosen places (meteorological stations) to gather information on the distribution in the main wind direction.
2. Trial of cascade impactors at the sampling places to evaluate ^{210}Pb-concentrations with reference to the particle diameter of the suspende matter.

III. Progress achieved:

1. Methodology

^{210}Pb: low level beta-counting preceding precipitate exchange on thin sulphide layers

^{226}Ra: alpha-scintillation using the decay of the daugther nuclide ^{222}Rn in equilibrium

Inactive elements (K, Ca, Ti, Ma, Fe, Zn, Rb, Sr, Y, Zr, Pb): energy dispersive X-ray fluorescence

U and Th: extraction with tributyl phosphate (TBP) and bis-(2-ethylhexyl) phosphoric acid (HDEHP) and measurement with EDXRFA

2. Results

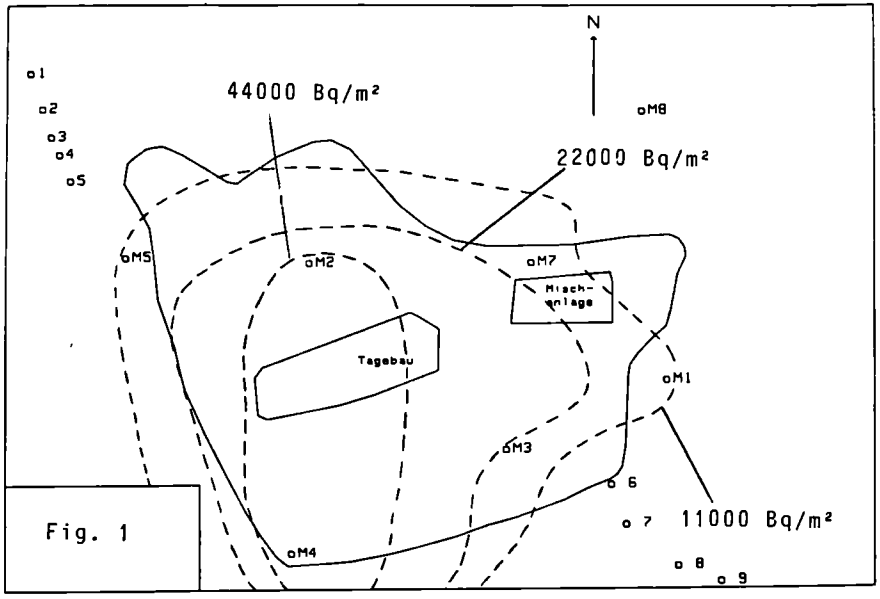
a)sampling period: June 29 - July 3, 1987: cascade impactor samples were taken at 6 meteorological stations, and soil samples were repeated. Additionally, two soil samoles were taken in the north and in the south of the uranium mining site taking into account geological criteria in order to determine the 'background'

b) enclosed are two figures on the deposition of ^{226}Ra and ^{210}Pb around the uranium mining site.

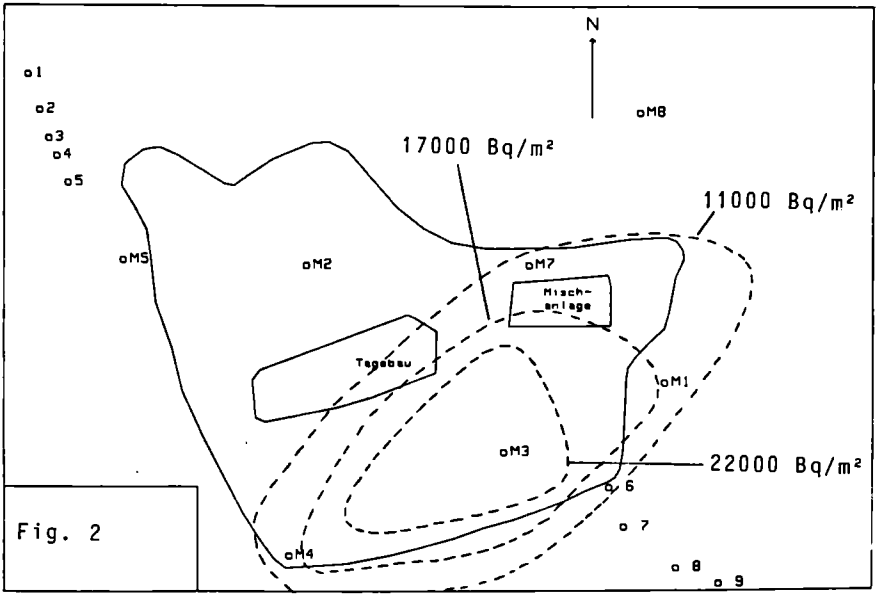
3. Discussion

Fig. 1: Deposition rates for ^{226}Ra are determinated by integration of the ^{226}Ra-concentration of the soil profiles (0 - 10 cm) and subtraction of the background (10 - 15 cm).

Fig. 2: ^{210}Pb- deposition was determined similar to ^{226}Ra. The difference of ^{210}Pb - ^{226}Ra gives the ^{210}Pb-deposition resulting from the ^{222}Rn transport and the decay into ^{210}Pb. The ^{210}Pb part from the ^{222}Rn transport is in agreement with the location of the waste air ducts and the resulting increased emission of ^{222}Rn.



Deposition of ^{226}Ra



Deposition of ^{210}Pb resulting from ^{222}Rn transport

IV. Objectives for the next reporting period:

Integration of Rn and U measurements and correlation with the existing results in order to achieve a better understanding of the transport and deposition mechanisms.

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

Kernforschungszentrum Karlsruhe
Postfach 3640
7500 Karlsruhe

Centre d'Etudes Nucleaires
60-68 Avenue du Général Leclerc
BP. n°6
92265 Fontenay-aux-Roses

VI. Publications:

No publications for this reporting period

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-032-UK

Imperial College of Science
and Technology
Exhibition Road
GB- London SW7 2AZ

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

Dr. J.N.B. Bell
Pure and Applied Biology Dept.
Imperial College Reactor Centre
Silwood Park
GB- Ascot, Berkshire SL5 7PY

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:

Dr. J.N.B. Bell
Department of Pure & Applied Biology
Imperial College, Silwood Park,
Ascot, Berkshire, SL5 7PY, U.K.

Miss M.J. Minski
Imperial College Reactor Centre
Silwood Park, Ascot,
Berkshire, SL5 7PY, 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:

Application of aerosols contaminated with radionuclides in wind-tunnel experiments on crops at different growth stages, followed by transfer to the field, including dual isotope experiments. Measurements of ^{137}Cs and ^{134}Cs on winter wheat growing in the lysimeters at intervals up to the final harvest; corresponding measurements of total exchangeable Cs in the soils and movement down the profile. Extension of the lysimeter experiments to other crops for the summer growing season.

III. Progress achieved:

1. Methodology

During the reporting period the wind-tunnel has become fully operational for the administration of labelled aerosols onto crops. This follows the characterization of the aerodynamic properties of the tunnel described for the previous reporting period, which used wooden blocks as surrogates for spaced plants. Experiments have been performed with grass swards, peas, young cabbage plants, and leeks, with measurements of aerosol gradients in the air above the crop and levels of contamination on the crop in each case, thereby generating data for velocities of deposition. The aerosol generation facilities installed at the upwind end of the tunnel have been upgraded by the addition of a Kr source for removal of electrostatic charges. Further improvements have been made to the aerosol sampling system and the soft-ware associated with the traversing gear used with the hot-wire anemometer, thereby resulting in significantly greater accuracy in air concentration measurements.

Unfortunately health physics considerations have precluded use of radioactive aerosols in the tunnel during the reporting period. However, in order to fulfill the objectives of the contract, a procedure has been developed to perform dual isotope experiments using ^{133}Cs (stable) and ^{137}Cs. This involves growing plants in nutrient solution in a Perlite matrix spiked with ^{137}Cs to provide the tracer for the soil-plant pathway. The ^{133}Cs is applied in 5μ silica aerosols onto the shoots in the wind-tunnel, with the Perlite protected from deposition, thereby providing a separate identifiable tracer for the direct air-plant pathway. The ^{133}Cs is measured by neutron activation analysis.

The lysimeters containing the soils characteristic of the main wheat growing areas of the CEC which now contain both ^{137}Cs (applied in 1983) and ^{134}Cs (applied in 1986) have been used during the reporting period for growth of three different crop species in order to determine soil-plant transfer factors for aged and fresh contamination. Winter wheat sown in 1986 has been harvested on three occasions up to maturity in September and counting of separate plant parts is in progress. During the summer 1987 growing season, spring oil-seed rape and savoy cabbages have been grown in the lysimeters, with sequential harvesting to maturity (September for the oil-seed rape and December for the cabbages). In the case of the final cabbage harvest, the roots have been separated from the soil for counting to determine total plant ^{137}Cs and ^{134}Cs uptake.

2. Results

Figure 1 shows the horizontal profile for aerosol ^{133}Cs concentration in the tunnel at 255 mm and 370 mm above a 24-day old spaced pea crop. This shows a highly satisfactory level of uniformity across the working section of the tunnel, particularly in the central region unaffected by the side walls.

Figure 2 shows the air concentration of Cs aerosol as measured at the end of the test section above and below the pea crop. This was constant to within 5% from 100 mm to 370 mm above the crop, with a sharp fall below canopy due to interception by the crop.

Fig. 1 - Integrated air conc. of Cs aerosol above pea crop

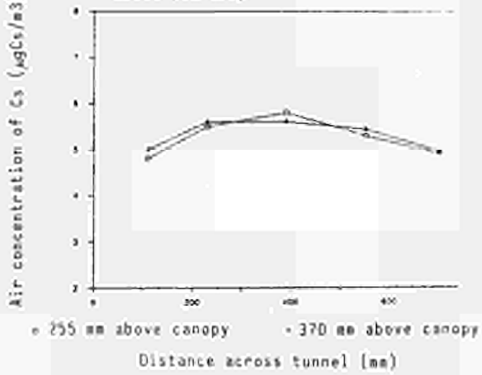


Fig. 2 - Integrated air conc. of Cs aerosol over pea crop

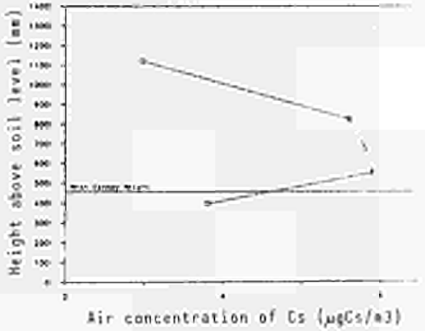


Table 1 - Preliminary gamma activity (Bq/g) and transfer factors ((Bq/g air dry plant)/(Bq/g air dry soil)) for 48 day old spring oil seed rape grown on eutric cambisol.

Tillage	Soil/Plant part	Cs-134		Cs-137	
		Activity	Transfer factor	Activity	Transfer factor
ploughed	soil	300	/	27	/
	stem	3.7	0.051	0.5	0.04
	petiole	3.4	0.07	[illegible] sample	
	leaf	4.5	0.041	0.8	0.01
not ploughed	soil	350	/	30	/
	stem	4.1	0.029	0.6	0.017
	petiole	4.2	0.028	0.94	0.023
	leaf	5.0	0.04	1.2	0.01

Table 1 shows preliminary results obtained for the first harvest (48 days old) of the spring oil-seed rape grown on a eutric cambisol. This indicates a high level of uniformity of distribution of both radionuclides within the plant. The most notable feature, however, is the surprisingly close similarity for the transfer factors for caesium which has been present in the soil for 4 years compared with less than one year. Furthermore, simulated ploughing appears to have had little effect on Cs uptake, contrary to results reported for wheat, using the same system. However, confirmation of these results depends on counting of the replicate samples, which is currently in progress.

3. Discussion

The experiments with the lysimeters are continuing on schedule and it is proposed during the next 6 months to deal with the backlog of counting and generate time-dependent transfer factors for all 3 crops. The wind-tunnel is now fully operational. Although it is still not possible to work with radioactive aerosols, the development of the $^{133}Cs/^{137}Cs$ technique will permit the dual isotope experiments to be performed fo fulfill the objectives of the contract.

IV. Objectives for the next reporting period:

Continuation of the lysimeter studies, using crop rotation with winter oil-seed rape, winter wheat, and cabbage. Counting of backlog of samples from 1987 experiments. Dual-is tope studies on the relative importance of soil and air to plant pathways for contamination of edible crop parts at different growth stages.

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

None

VI. Publications:

1. Grogan, H.A., Mitchell, N.G., Minski, M.J. and Bell, J.N.B. (1987). Pathways of radionuclides from soils to wheat,In: (P.J. Coughtrey, M.H. Martin and M.H. Unsworth, eds.). Pollutant Transport and Fate in Ecosystems. pp 353-70. Blackwell Scientific, Oxford.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 windtunnel

Head(s) of project:
Prof. Dr. H. Bonka
Lehrgebiet Strahlenschutz in der Kerntechnik der RWTH Aachen
Templergraben 55, D-5100 Aachen

Scientific staff:
Dr.-Ing. H.-G. Horn; Dipl.-Ing. M. Maqua;; Dipl.-Ing. D. Oberschachtsiek;
Dipl.-Ing. Th. Hattingen

I. Objectives of the project:

- Measurement of the vertical distribution of eddy diffusion-coefficients in and above different kinds of vegetation (field and windtunnel experiments)
- Use of the results of the measurements to improve the models for the calculation of the dry deposition of radionuclides bound to aerosol particles and radioiodine

II. Objectives for the reporting period:

- Continuation of the field experiments with additional measurements of the eddy flow
- first wind-tunnel experiments with model vegetation
- development of a computer code for the transformation of the measured three dimensional eddy-flow data to eddy-diffusion coefficients

III Progress achieved

1. Methodology:

- Wind-tunnel modelling of the exponential profile above ground in 1:1 scale
- Comparison of modelled turbulence with turbulence measured in the field
- Design of 1:1 scale model-grass and installation in the wind tunnel
- Vertical wind-speed and turbulence measurements in and above the model grass vegetation
- Development of FFT-techniques to analyse frequency distribution in turbulence
- First analysis of one dimensional flow recording with FFT

2. Results

The caracteristical flow over bare ground as well as the profile within the grass vegetation were modelled in the wind tunnel. Comparison with field measurements showed a good agreement of the $\bar{u}$-profile as well as the turbulence characteristics (see Fig. 1).
To model the grass canopy, brass strips (0.1 x 4 x 100 mm) were irregularly fixed in a styrofoam bed. The flow above bare ground could be modelled in the first 1.3 m of the test section (Fig. 2). The following model-grass canopy has a total length of 1.2 m. It can be extended to a total length of 1.7 m.
Spatial equilibrium conditions in flow through the grass are reached after approx. 0.8 m.
The wind speed profiles were recorded at different $\bar{u}$ above the grass. As an example, Fig 3 shows the flow and the result of a FFT analysis at z - 5 cm in the grass for $\bar{u}(z = 27\ cm) = 3$ m/s.

In order to analyse the three-dimensional flow, the calibration techniques for 3-wire-probes were tested. Also, programmes were developed to analyse the frequency distribution in the flow as well as correlations between the three components of flow.

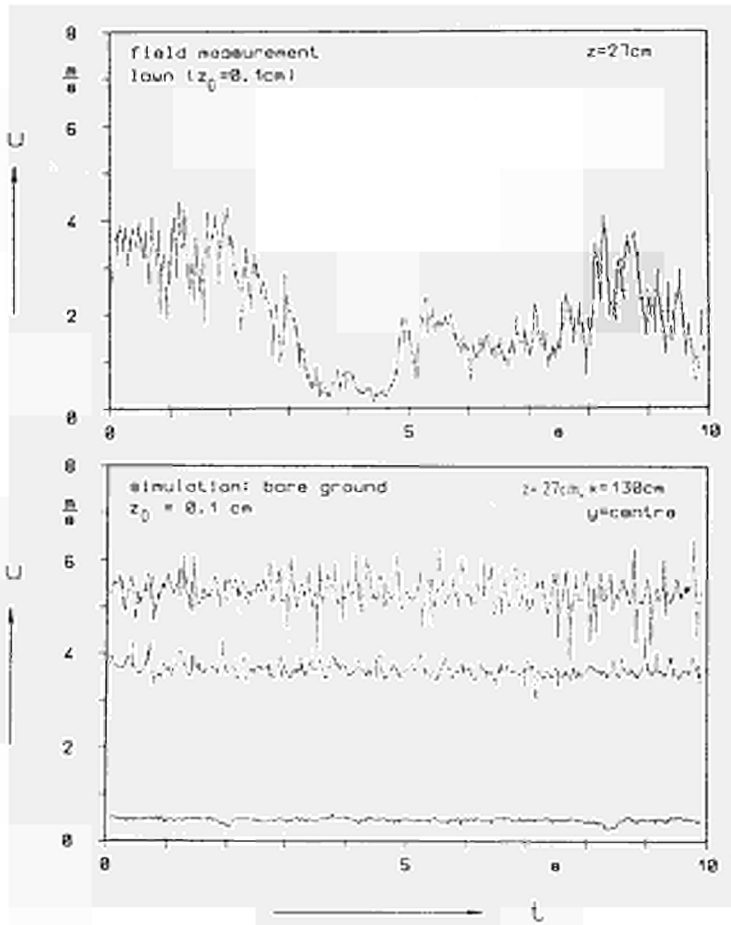


Fig. 1: Mesured flow in the field and in the wind tunnel

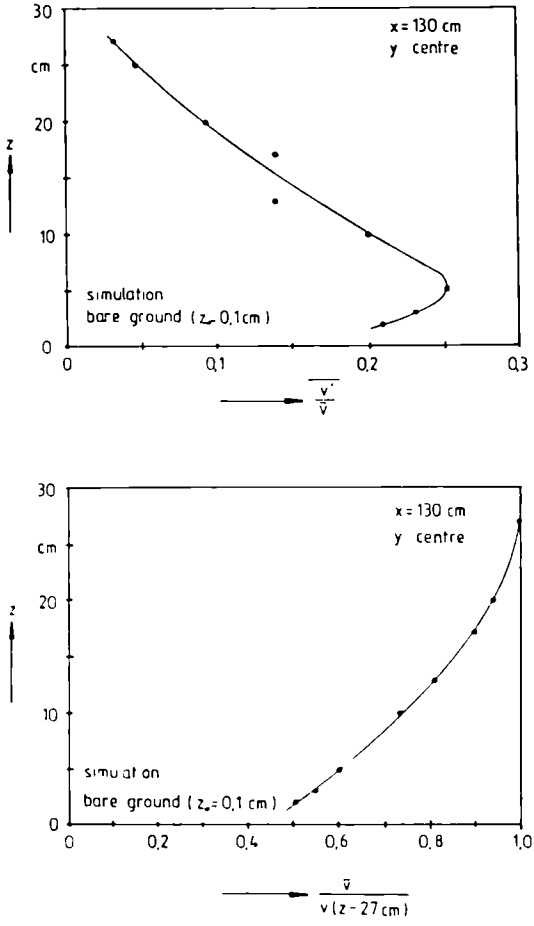


Fig. 2: Flow characteristics in the wind tunnel

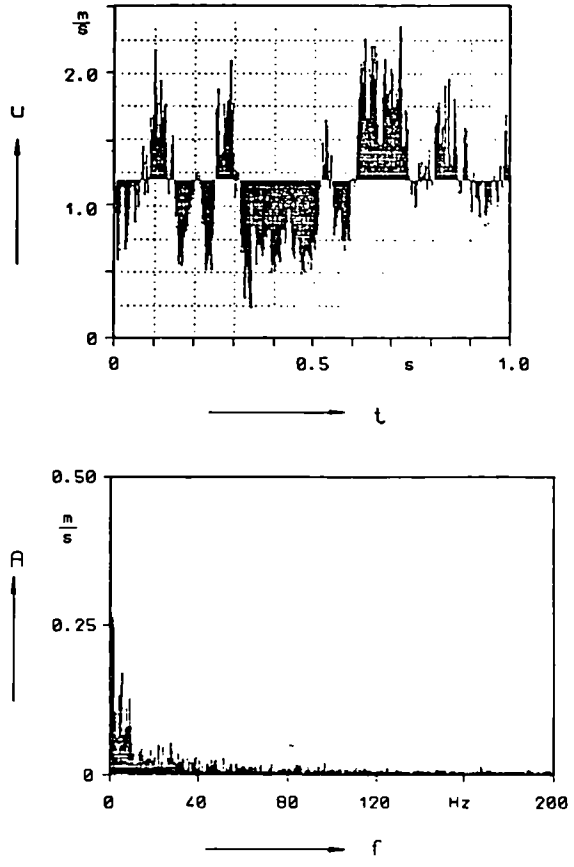


Fig. 3: Flow and frequency-analysis in the modelled grass (h = 10 cm) at z = 5 cm, u (z = 27 cm) = 3 m/s

3. Discussion

All experiments made during the last year showed that it is possible to model the flow within and above the grass vegetation sufficiently compared with the field. The wind-tunnel model installed is a good base to proceed with three dimensional measurements of flow and turbulence in the next reporting period.

IV. Objectives for the next 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.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-055-D

BRENK Systemplanung
Heinrichsallee 38
D-5100 Aachen

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. Extended literature search
2. Acquisition and compilation of appropriate material and data-bases
3. Development cf the hydrological model
4. Development cf a surface specific erosional model
5. Physico-chemical processes affecting the sorption/ desorption behaviour

II. Objectives for the reporting period:

Reporting period: 01.01. - 31.12.1987

1. Extended literature search
2. Acquisition and compilation of appropriate material and data-bases
3. Development of the hydrological model

III. Progress achieved:

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 hydrological characteristics of such surfaces.
The considerations are intended to form the basis of a modeling effort to address the specific needs of regional type accident consequence analysis problems.

2. Results

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 al. (1):

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. At first the hydrological characteristics of urban surfaces will be discussed below.

3. Discussions

The description of the hydrological characteristics of urban drainage areas including pervious and impervious surfaces is a fairly well established scientific subject and has been documented in many publications and standard textbooks, cf. CHOW (2), DYCK et al. (3). In the context of this study the establishment of rainfall-runoff-infiltration relationships is of primary interest.
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 the media of the drainage system as well as the climatic and physiographic factors involved. The most important parameter appears to be the infiltration capacity of the surface which is largely determined by the soil texture, thickness, degree of compaction, and the soil moisture content. Impervious surfaces, by definition, have a zero infiltration capacity.
A variety of models has been devised in the literature for estimating the surface runoff volume connected to the sewage system including the rational formula, linear and nonlinear reservoir models, the unit-hydrograph theory, the runoff-curve number approach, etc.. For describing the rainfall-runoff characteristic of urban impervious surfaces a simple linear reser-

voir model described by NEUMANN et al. (4) has been adapted for this study. This model relates the surface runoff (R) to the amount of precipitation (P), the depression storage (M), and the loss by runoff to pervious surfaces (V) of a storm event and is given by $R = P - M - V$ (R,P,M,V given in mm).

Based on experimental studies in urban environments, Munich, West Germany (4), a value of M = 1 mm and a precipitation rate dependent loss V (on the order of 0 - 15 % of the rainfall) has been adopted for this study.
For pervious surfaces a rainfall-runoff relationship similar to the equation mentioned above may be used. However, appropriate allowance has to be made for the infiltration capacity of this type of surface. Numerous equations are available in the literature for describing the infiltration capacity of a soil, incl. the HORTON-equation (5).
Hydrometeorological data of a typical West European site along with an increased depression storage (M = 3 mm) of soil surfaces indicate that surface runoff of this type of surface is relatively seldom to occur, unless that local factors promote the formation of surface runoff.

(1) DONIGIAN, A.S. et al., in: Modeling of Rivers (ed. by SHEN, M.W.), Chapter 12, J. Wiley and Sons, New York 1979
(2) CHOW, V.T. (ed.), Handbook of Applied Hydrology, Mc Graw Hill Book Comp., New York 1964
(3) DYCK, S. et al., Grundlagen der Hydrologie, W. Ernst Verlag, Berlin 1983
(4) NEUMANN, W. et al., Berichte aus Wassergütewirtschaft und Gesundheitsingenieurwesen, Institut für Bauingenieurwesen, TU München, Nr. 11 (1976)
(5) HORTON, R.E. Transactions of the AGU, No. 20, (1939)

IV. Objectives for the next reporting period:

1. Extended literature search
2. Acquisition and compilation of appropriate material and data-bases
3. Development of a surface specific erosional model

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-035-B

Katholieke Universiteit Leuven
KUL
Naamsestraat 22
B-3000 Leuven

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 scavenging and remobilization processes. The various factors which are being considered are the geochemical composition, physico-chemical conditions and microbiological effects.

II. Objectives for the reporting period:

The main activities for the 1987 reporting period were directed towards the following objectives.

- The geochemical phase associations of Eu in soils and sediments
- The behaviour of Zn-65 in soils. Particular emphasis is placed on the identification of retention mechanisms and the competitive effects between different adsorbents.
- Technetium retention and reoxidation rates of reduced Tc-forms in soils.
- Quantitative and interpretative study of the solid/liquid distribution behaviour of radiocesium in soils.

III. Progress achieved:

Europium geochemical phase association in soils

In order to assess quantitatively the geochemical phase associations of lanthanides, a quantitative characterization is neccesary of the binding constants of the different potential sinks in the soil or sediment. A comprehensive study was made on the stability constant of Eu with humic acids (HA) of different origin (commercial, and various subfractions of podzol HA). On the basis of an ion exchange method, developed in this laboratory, it was shown that the stabilities of the Eu-HA complexes were remarkably similar for all samples : Log K = 14±.5. Moreover, it was shown that in dilute solutions, a 1-1 Eu-HA complex is formed and that transition metals were poorly competitive with Eu complexation.

Humic acids : quantitative characterization

The experimental study of the binding of radionuclides by humic acids requires methodologies for measuring the nature and capacities of the functional groups involved in complex formation. Two methodological developments have taken place during the 1987 period.

1. Titrimetric characterization

Acidimetric titrations were carried out on a series of HA of different origin, making use of an automated and completely informatized titration system at the chemistry dept. (Dr. R. Paterson) of Glasgow University. This procedure allows the identification of various functional groups by plotting the titration data differentially in terms of the pH change per unit added base or acid. In general, two or three peaks, corresponding to different functional groups can be identified. Figure 1 shows such a plot for a podzol-extracted HA, characterized by functional groups which are titrated at characteristic pH values of resp. 4.25, 7.22 and 9.73.

2. The cobalti-hexammine method

A new method has been developed for measuring the functional group capacity of HA. either in a mixed sediment or soil or a very dilute HA extract. The method is based on the strong coagulating effect of cobaltihexammine (Cohex) on HA : the addition of an amount of Cohex, equivalent to the functional group capacity leads to quantitative HA coagulation. Analysis of the pH-dependence of the functional group capacity, obtained by using Co-60 labelling, clearly demonstrates the occurrence of the two functional groups (carboxylic, phenolic) : the capacity of the COOH-groups, obtained by this method, agrees with the result obtained by the classical Ca-acetate method. On the basis of this method, it could be shown that the podzol extracted HA is significantly enriched in phenolic groups, as compared to the bulk soil.

Behaviour of Zn-65 in soils

The key mechanisms, governing the retention of trace quantities of Zn in soils are complexation with oxides and humic acids. Competitive effects for Zn sorption were studied for a podzol (HA system) and goethite, a typical oxide mineral. The Zn sorption in the separate

systems showed pronounced differences in pH dependence : both systems can be simulated succesfully in terms of multiple-site adsorption models. For the mixed systems, it could be shown that in fairly acid conditions, HA is the dominant sink. At a pH of about 5, the oxide becomes the phase controlling the Zn sorption. A typical example of the behaviour of Zn in a mixed system is shown in figure 2.

Technetium

Two issues were addressed during the 1987 period : the distribution of reduced forms of Tc between fulvics and humics and the effect of alternating oxic and reducing conditions on the reoxidation of Tc. In podzol extracts a tendency could be demonstrated for a Tc enrichment in the fulvic acid fraction.
Preliminary findings further indicate that alternating redox conditions are conducive to the formation of less readily oxidizable Tc-forms, a finding which is relevant to the aging effect of Tc in soils.

Cesium retention in soils : quantitative aspects

1. Current views

There is a general consensus that the single most important factor, determining the Cs retention in soils and sediments is the property of certain layer silicates (hydrous mica's) to preferentially adsorb alkali ions of low hydration. However, due to the lack of quantitative insight into this interception process, it has not been possible to assess these phenomena in terms of their effect on the solid/liquid distribution behaviour of Cs in the system. Some significant methodological developments have occurred within the context of another CEC program (Nuclear contamination of the urban environment) which enable us to quantify this effect.

2. Methodology and results

The principle of the method is based on the use of cationic complexes such as silver thiourea and cobaltihexammine which exhibit very high selectivity for the regular clay exchange sites but which, for steric reasons, are excluded from the Cs-high affinity sites (HAS) in hydrous mica's. Thus in the presence of a suitable background level of such "masking agents" it is possible to quantitatively characterize the Cs specific sites in terms of their number and their Cs-selectivity. This procedure was tested for a loam and an clay soil which showed HAS capacities of resp. 0.12 and 0.27 meq./100 g. Moreover, the Cs to K selectivity patterns on the HAS were found to be convergent, as illustrated in figure 3, showing the composition dependence of (Ln)Kc of Cs to K. Consequently, it appears that different soils may be characterized by a common quantitative characteristic as regards Cs sorption. It follows that the in situ solid/liquid distribution behaviour of Cs in soils can be expressed quantitatively in terms of the capacity of the HAS, the Cs-K selectivity coefficient and the K concentration in the soil solution. The quantitative relation has the form

$$K_D = \frac{(HAS)\ K_c\ (Cs\text{-}K)}{m_K}$$

Consequently, the K_D(Cs) value, the key parameter in the soil-to-plant transfer, can be rationalized and predicted in terms of readily measurable soil properties.

Methodology for in situ K_D measurements in soils

In order that radionuclide K_D values be relevant to the quantitive assessment of soil-to-plant transfer, it is mandatory that they refer to conditions which are representative for the "in situ" field condition. A method is developed which is based upon the disperison of a soil sample in a (Ca+Mg) solution of resp. 5 and 10 meq/l, i.e. the range of ionic strengths normally occurring in soils. Such method generates K and Ca+Mg levels in the equilibrium fluid which are in the range found in the soil solution by immiscible displacement techniques. In the particular case of Cs and Sr, it is shown that respectively K and Ca + Mg liquid phase levels the are key factors for the in situ distribution behaviour of these radionuclides.

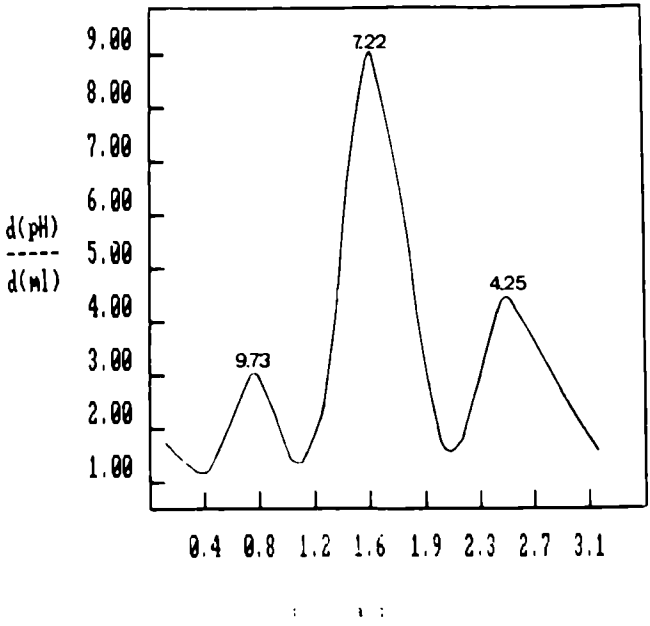


Figure 1. Differential titration curve for a podzol-extracted humic acid.

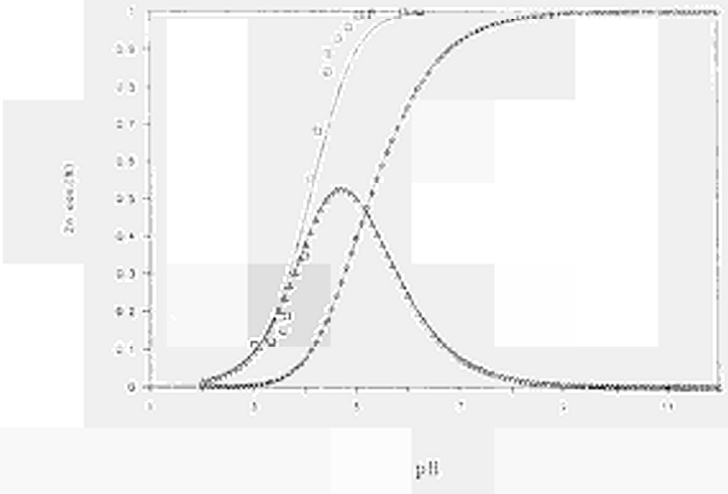


Figure 2. pH effect on Zn sorption in a 1/4 goethite podzol mixture (□ exp.; —— simulation) and the fractional distribution in the mixture (◇ podzol; ◇ goethite).

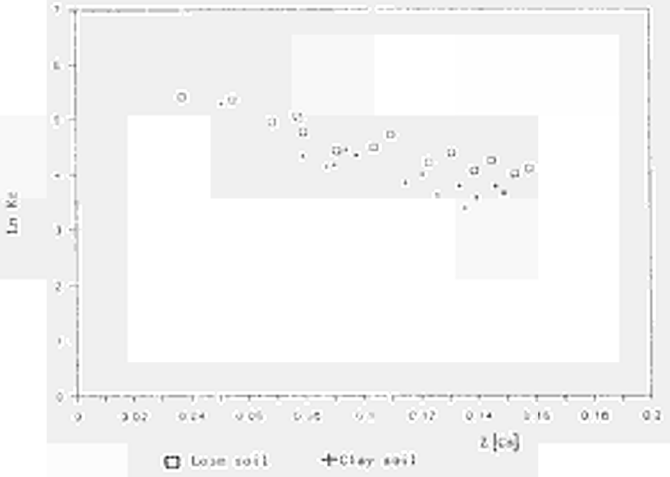


Figure 3. Selectivity coefficient (Ln Kc) of Cs to K in high affinity sites in a loam soil and a clay: ln Kc tends towards a value of about 6 at vanishing Cs loading Z(Cs).

IV. Objectives for the next reporting period:

- Quantitative and interpretative study of cesium retention in soils under in situ conditions.
- The humic acid chemistry of technetium and Tc-aging phenomine in soils.
- The geochemical phase association of Zinc-65 in soils.
- Humic acid chemistry of Europium.

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

- Laboratoire de Biochmie, Université de Nantes (P. Pierl)
- Chemistry Dept. (R. Paterson), Glasgow University.

VI. Publications:

- Maes A., De Brabandere J. and Cremers A.
  A modified Schubert Method for the measurement of europium humic acid complexes in alkaline conditions.
  Radiochemica acta (in press).
- Maes A., Henrion P., De Brabandere J. and Cremers A.
  Europium humic acid complexes in reducing conditions. (Published as abstract).
  Int. Conf. Chemistry and migration of activation and fission products in the geosphere. Munich, Sept. 1987.
- Stalmans M., De Brabandere J., Van Elewijck F., Maes A., Cremers A., Henrion P. and Coqneau M.
  The fate of technetium in soils and sediments : A physico-chemical approach.
  Health Physics (in press).
- Stalmans M., Maes A., Sterckx D., Cremers A. and Cogneau M.
  Technetium organic matter associations in soils : Methodological aspects.
  Health Physics (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Nuclear Energy Board
3 Clonskeagh Square, Clonskeagh Rd.
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 Rd.
IRL- Dublin 14

Telephone number: (1) 83.83.56

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 transfer factors for various types of agricultural produce. The transfer factors 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 and through a detailed examination of the soil characteristics.

II. Objectives for the reporting period:

The first objective was to select a number of representative locations typifying the range of different soils used for agricultural purposes in Ireland. At each of these locations soil samples would be taken on pasture land and tillage land. Sampling of soil would be carried out twice yearly. Samples of grass would be taken simultaneously, with an additional sampling round for grass in the intervening period. Agricultural produce would be sampled just prior to harvest.

III. Progress achieved:

Methodology

Eleven sites were selected for sampling purposes. The locations were chosen so that the principal soils used for agricultural purposes in Ireland were represented. Where possible, the sites were located in areas which had suffered high deposition following the Chernobyl accident.

With regard to the agricultural produce, it was decided to concentrate on three main types - wheat, barley and potatoes. These were expected to be widely available and this would therefore enable comparison of results. Some additional vegetable samples would be collected where available, particularly in areas which concentrated on vegetable rather than cereal production.

Following selection of the sites, sampling commenced in Spring 1987. The sampling methods were decided in advance of the first sampling round and were essentially unchanged during further rounds. They may be described as follows:-

(i) Soil from the pasture land was taken using 4 corers, of decreasing diameters, one for each of the depths 0-5 cm, 5-10 cm, 10-15 cm and 15-30 cm. The cores were taken at 25 points scattered over an area of approx 100 m^2 and bulked to form 4 samples, one for each depth. The soil was oven dried and sieved to 2 mm.

(ii) Sampling of tillage land was carried out using a single 0-20 cm corer. This depth was thought to correspond to the ploughing depth in most areas. A total of 20 cores was taken from an area of approx 100 m^2. Sample preparation was as for the pasture soil.

(iii) Grass samples were cut from a height of approx 5 cm above the ground. The grass was then air dried before being ashed.

(iv) Cereal samples were taken, a few days before they were due to be harvested, from 1m x 20cm plots scattered over an area of approx 100 m^2. The samples were thrashed and the grain was ashed.

(v) Potato samples were picked from the site which had earlier been sampled for soil. The potatoes were scrubbed clean prior to ashing.

Sampling for the first year was concluded in October 1987. All soil samples have been tested for particle size, C.E.C., total carbon content and available potassium. Gamma spectrometric analysis has been carried out on the pasture land soils collected in the first round of sampling. Measurements on cereal and potato samples have also been completed.

Results

Analysis of the pasture land soil has shown that the most abundant radionuclides present as a result of Chernobyl are Cs-137, Cs-134 and Ru-106. Examination of the core profiles from the pasture land indicates that, one year after Chernobyl, most (>80%) of the deposited radiocaesium and Ru-106 is retained in the first 5 cm of soil. It is also clear that there is a significant contribution (between 8 and 40%) from weapons fallout to the amount of Cs-137 present at this depth. Transfer factors between soil and grass will be calculated when analysis on the grass samples has been completed.

Measurements on cereal samples indicate that levels of Cs-137 in both wheat and barley are of the order of 0.1 Bq kg^{-1}. As results on the tillage soils are not yet available, the transfer factors can not yet be calculated.

Measurements on levels of Cs-137 in potatoes range from .02 Bq kg^{-1} to 2.1 Bq kg^{-1}. The highest levels were found in potatoes grown in a peat organic soil. Some additional vegetable samples collected at this site also showed relatively high levels of activity.

Discussion

Conclusions on the effect of soil characteristics on the transfer of Cs-137 between soil and plant must await the results of the activity analyses on the tillage soils and on the grass. However it is already clear that the organic matter content is important with respect to the absorption of Cs-137 by plants. It would appear that this nuclide enters plants more readily from soil rich in organic matter than from those which are predominantly mineral in character. Particular attention will be paid to this site in the sampling programme for the second year.

Comparison of data from pasture land cores from different sites shows a variation in rate of movement of Cs-137 and Cs-134 down through the core. The extent of downward movement is determined largely by the degree of exchange on adsorption to mineral particles and also by the amount of water movement. An attempt at correlating the rate of vertical movement of radiocaesium with soil characteristics will be carried out when the analysis for further sets of pasture samples has been completed. The rate of downward movement of Cs-137 has important consequences for the amount available for uptake by grass.

The sampling programme for the second year of the project will be finalised when measurements on the first years samples have been completed. It is envisaged that sampling will be continued at all of the eleven sites. However, the results of the first sampling round may indicate that it would be most beneficial to the project to concentrate resources at certain sites.

IV. Objectives for the next reporting period:

The first objective is to complete measurements on all samples collected during the first year of sampling. Analysis of the results of these measurements will give the first estimates of transfer factors.

Sampling will begin again in Spring 1988 and should be completed by October 1988. Measurements on these samples will commence immediately after collection and are expected to be completed by Spring 1989.

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

An Foras Taluntais
Johnstown Castle Research Centre
Wexford
Ireland

Physics Department
Trinity College
Dublin 2
Ireland

VI. Publications.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: B16-B-034-I

Comitato Nazionale 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)]:

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.: BEHAVIOUR OF Tc AND Se IN THE MARINE ENVIRONMENT.

Head(s) of project:

Scientific staff: SCHULTE E.H., PAPUCCI C., R. DELFANTI

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 committments 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 impact on man.

II. Objectives for the reporting period:

- macro and microdistribution of selenium in benthic organisms
- studies on the vertical transport of radionuclides in the water column.

III. Progress achieved:

Studies on the vertical transport of radionuclides in the water column.

Once introduced into superficial ocean waters, radionuclides are dispersed by physical and biological processes such as currents and transfers through food chains. Besides the horizontal dispersion, mainly exerted by currents, the vertical trasport of radionuclides from superficial waters to the deep ocean and to bottom sediments represents an important pathway and input of radioactivity to sediments which may act as the ultimate sink for different radio-isotopes.

The event of the Chernobyl nuclear accident and its radioactive fall-out presented a unique scientific opportunity to study the geobiochemical behaviour of short- and long-lived radionuclides in the marine environment after a single pulse input.

The field and laboratory investigations, started in 1986, intended to evaluate biologically mediated processes in the water column in transporting radionuclides as well as their deposition on the sediment surface and the biological importance (bioturbation) in phenomena which lead to the incorporation of radionuclides into the sea-bottom and to their remobilization from anoxic sediments.

In field studies radiotracers delivered by the Chernobyl accident were considered, while in laboratory experiments radioisotopes of Se and Tc were used.

Following the Chernobyl accident, measurements on radioactive fallout and selected marine samples were carried out. In the Ligurian Sea peak concentrations of Chernobyl radionuclides in surface seawater were found on day 4 and 5 of May 1986. After three weeks of contact with seawater only 2% of 137-Cs and 5% of 103-Ru present in the water column were in the particulate form and could be trapped on 0.45 μm filters.

Since ruthenium normally occours in the particulate state or adsorbed to particulate matter, most of the fallout ruthenium should have been in the particulate form at the time of immission into the seawater, while cesium isotopes are most soluble and dilute readily in the water column.

This fact is reflected in the different behaviour of Cs and Ru in samples of Mytilus sp. measured for radioactivity every second day from May 2nd onwards. Maximum concentrations of cesium (7.7 Bq kg^{-1} FW) occured in mussels only one day after the revealed peak concentration of Cs in seawater, as to be expected due to its high solubility, and dropped sharply during the following days reaching values close to normal background concentrations (0.2 Bq kg^{-1} FW) two months later.

On the contrast the behaviour of ruthenium isotopes showed a marked delay with respect to the cesium isotopes.

Maximum concentrations (235 Bq kg^{-1} FW) for 103-Ru were observed in mussels three days after the peak concentration in the water and sharply decreased over the absorbition period (56 days). This fact revealed the settling and/or sedimentation behaviour of particulate ruthenium which reached mussels some 4-5m below the surface with a delay of 1-2 days with respect to the more soluble cesium.

In coastal areas ruthenium isotopes reached shallow water sediments quite rapidly. At the beginning of June 1986 most of the radioactivity in the upper sediment layers was due to ruthenium isotopes while in July 1986 no radionuclide from Chernobyl fall-out could be detected in sediments from 500 m depth. Either the sedimentation velocity of particulate ruthenium was too slow or the formation of the thermocline impeded the passage of ruthenium particles to deeper waters.
On the other hand the vertical transport processes for cesium were much slower. Only 6-12 months after immission about 10% of cesium isotopes deposited at the sea surface were found in the upper 2-4 cm of shallow water sediments; i.e., thus about 90% of the cesium of the Chernobyl fallout is still solved in the water column and will be transferred to sediments over longer time scales.

Studies on the remobilization of Selenium from sediments by biological activities (bioturbation)

Investigations on the influence of the infaunal biological activities on the biogeochemical cycling of radionuclides were continued, considering the behaviour of different physico - chemical forms and compounds of selenium-75 at the sediment-water interface.
As demonstrated in previous studies, bottom-dwelling organisms rework the upper sediment strata (20-30 cm) and re-oxidize portions of anoxic sediments, thus previously reduced and immobilized radionuclides may be re-oxidized and re-cycled to the water column.
Aliquots of a strongly reducing coastal sediment sample were mixed with de-oxigenized seawater to gain a slurry to which then different chemical forms of 75-Se were added under strict anoxic conditions. One aliquot (400 g) was spiked with 144.3 kBq of 75-Se-methionine, a second aliquot (500 g) with 85.8 kBq of 75-Selenite, and a third one (450 g) was spiked with 91.6 kBq of 75-Selenate. The slurries with 75-Se were stirred for more than one hour sustained by vigorous bubbling of nitrogen through the suspension and then filled equally into six tubes, two tubes for each 75-Selenium form (Ø 3.2 cm; 35cm lenght; surface 8 cm^2) and let settle for 10 days. Only one tube of the three pairs of tubes for each 75-Se-form received specimens of the polychaete worm Marphysa bellii weighing 1.12; 2.65 (75-Se-Methioline), 2.40; 1.35 (75-Selenite) and 1.70; 1.85g (75-Selenate), while the remaing three tubes served as blanks. The whole oxidized overlaying water volume was sampled daily and measured for radioactivity.

After sufficient settling of the sediments but prior to the start of the experiments, the radioactivity in the overlaying water was determined. The measurements revealed that 96.5; 97.5; and 80.3% of the spiked radioactivity of 75-Se-methioline, 75-Selenite, and 75-Selenate were bound to the sediments.

After 73 days only very small fractions of the radioactivity of the different Se-forms were remobilized from the sediments by the worms and realesed to the water column and/or subsequentely to the air where they were trapped by active carbon.

Total remobilized fractions of radioactivity from Se-methionine, selenite and selenate contamined sediments amounted to 9.57; 6.61; and 6.13%, respectively. Of these percentages parts of 4.8; 3.1; and 3.3% were lost to the water, while the blanks without the worms' activity lost only between 0.62 - 0.73% of the total radioactivity present in the sediments, probably by diffusion processes. All blanks showed a distinct reoxidized sediment zone of 7 mm depth at the end of the experiment.

In all three experimental sets volatile forms could be detected and trapped on active carbon (Se-Met: 1.22; Selenite: 0.66; and Selenate: 0.79%). The contents of the different Se-forms in the sediment-dwelling worms were surprisingly low; only 2.92; 2.67; and 1.93% of the initial Se-methioline, selenite, and selenate, respectively, were accumulated in the polychaetes. Concentration and transfer factors will be calculated when stable Se-measurements are available.

At the end of the experiment the sediments contained still 70-86% of the initial radioactivity according to the Se-forms. After centrifugation the radioactivity in interstitial waters of the sediment samples varied between 0.05 - 0.25% of the initial activity thus, it can be assumed that at the beginning of the experiment all radioactivity was bound to the sediment. Interstitial water contents of the sediment varied on a weight/weight basis from 17.7-to 21% in the presence of worms and from 36.1-37.6% in the blanks, demonstrating clearly the effectiveness of the biological activity of the polychaetes in strongly compacting superficial sediments.

Preliminary experiments made with aliquots of 75-Se-spiked water samples revealed high adsorption affinities of Se-forms to container walls in relative short time scales.

An average of 20% of the initial radioactivity was absorbed to the container walls.

Comparing the results to those of experiments made with Tc-95m under similar conditions it can be concluded that Se-forms are far more tightly bound to sediments than pertechnetate and consequently, their remobilization and recycling to the water column is a much slower process.

IV. Objectives for the next reporting period:

- macro and microdistribution of Se in benthic organisms
- role of zooplankton in vertical transport processes of radionuclides in the water column
- analyses of Tc-99 in biological matrices form the Mediterranean.

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

VI. Publications:

Caratteristiche del fallout da Chernobyl nell'ambiente marino costiero italiano:
Delfanti R. and C. Papucci; Proceeding of the III Conv. Naz. SITE, Siena 21 - 25/10/1986; in press.

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 recomandations 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:

- Role of mixing and sedimentation processes in 239,240-Pu redistribution in coastal marine sediments.
- Sedimentation and biodiffusion rates in deep-sea sediments.

III. Progress achieved:

METHODOLOGY

In 1983 and 1984 two sampling campaigns were carried out in the Gulfs of La Spezia (Ligurian Sea) and Gaeta (Tyrrhenian Sea) for studies on Plutonium behaviour in coastal marine environments. One core from each area was selected for the determination of the recent sedimentation rate and of the mixing characteristics. Both cores were collected in areas under the direct influence of a river (Magra river for the La Spezia Gulf and Garigliano river for the Gaeta Gulf).

In summer 1987 a sampling campaign was carried out in the Taranto Gulf (Ionian Sea). Sediment cores were collected along the slope of a canyon (Taranto Valley) and on the continental shelf, in areas characterized by the sedimentation of fine material.

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 first two areas and in coastal environments and by a SIPAN corer with subsequent subsampling with a core barrel in the last two areas.
The cores were sectioned directly onboard into slices 1 cm thick. Selected sections of each core were analyzed for 239,240-Pu, 210-Pb 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.
210-Pb determination was based on the measurement of its granddaughter, 210-Po, which is assumed to be in secular equilibrium with 210-Pb. The supported 210-Pb fraction was determined by the measurement of 226-Ra in several samples, using the Radon emanation technique.
14-C measurements were carried out using a benzene liquid scintillation counting method.

RESULTS AND DISCUSSION

a) Shallow water environment

The use of the vertical profiles of both 239,240-Pu and 210-Pb allowed the calculation of the sedimentation and mixing rates at the two sampling stations. A time dependent sedimentation-mixing model was used, based on a mass balance equation. The shape of radionuclide vertical profiles indicated, at both sites, low mixing rates. It was assumed a constant flux for 210-Pb and a 239,240-Pu input function

proportional to 90-Sr deposition. As the mixing rate seemd to be low, the mixing coefficient was assumed to be constant along the sediment column. In this way, sedimentation rates of 0.50 and 0.45 cmy-1 and mixing coefficients of 0.25 and 0.50 cm2y-1 were calculated for the La Spezia and Gaeta cores respectively. The shape and width of the Plutonium subsurface peaks and the 210-Pb vertical profiles were well described by the model, but the computed 239,240-Pu activities in the upper sediment layers were lower than the experimental values.
A higher mixing coefficient (in the order of 5 cm2y-1) could better simulate Plutonium profiles, but 210-Pb vertical distribution indicated, in both cases, low mixing rates.
A possible explanation of this fact is that particle supply by river runoff has increased the Plutonium flux to the sediments. In fact, at both sites 239,240-Pu inventories (La Spezia Gulf:130 Bqm-2; Gaeta Gulf: 255 Bqm-2) are much higher than the cumulative fallout deposition (82 Bqm-2) at these latitudes.

b) Deep sea sediments.

Sediment cores from deep sea sediments were collected from different sedimentary environments (abyssal hills, plain, fault areas). The Plutonium inventory calculated for the dumpsite area (14 Bqm-2) is comparable to those calculated for the NOAMP and GME hill stations (12 Bqm-2 and 9 Bqm-2 respectively) which are 13-20% of the total fallout delivery at the corresponding latitudes. In the SNAP area Plutonium inventories (0.3-1.1 Bqm-2) are only 1-3% of the fallout deposition. A higher inventory (36% of Pu delivery) was found in a NOAMP station located at the foot of an abyssal hill. In general, Plutonium inventories are strongly related to primary production. This factor can explain the low inventories calculated in the oligotrophic SNAP area, but it can not explain the differences between the two samples collected from the top and the foot of the same abyssal hill (NOAMP samples). 14-C measurements indicate undisturbed sedimentation (3.6 cmky-1) in the sample at the foot of the hill, with a mixed layer of 6-8 cm thickness. The sample collected at the top of the hill shows, on the contrary, a disturbed sedimentary record and higher 14-C age of the mixed layer. A possible mechanism leading to the unusually high inventory at the foot of the hill could be the transport of surficial eroded material downslope the abyssal hill, increasing Plutonium deposition at the foot.

The Plutonium vertical profile at the GME station shows a subsurface maximum and cannot be simulated by a classical biodiffusion model. For the description of the mixing characteristics at this site a model has been used that simulates the transport of surface, radionuclide-rich material to a fixed depth into the sediment by benthic organisms or an equivalent mechanism. By this model, a mixing coefficient of 0.1 cm2y-1 with a tranport rate of 0.1 y-1 was calculated.

IV. Objectives for the next 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.

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

VI. Publications:

Papucci,C. & Delfanti,R. (1987) - Distributions of 239,240-Pu and 14-C in North-East Atlantic sediments. Proceedings of the International Symposium on Radioactivity and Oceanography, Cherbourg, June 1-5, 1987. In press.

Delfanti,R., Buffoni,G., Papucci,C. and Paganin,G. (1987) - Determination of recent sedimentation rates in the Tyrrhenian Sea using 210-Pb and 239,240-Pu. Ibid. In press.

Papucci,C. & Delfanti,R. (1987) - Evaluation of particle mixing depth and rates in Atlantic surface sediments using 239,240-Pu vertical profiles. ESOPE cruise Report, JRC Ispra. In press.

TITLE OF THE PROJECT NO.: 3

DESCRIPTIVE MODELS FOR:
A) Circulation of radionuclides in the marine environment:
B) Transfer of radionuclides in the marine food-chains relevant to man.

HEAD(S) OF PROJECT: G.C. BOERI

SCIENTIFIC STAFF: M. BELLI, R. DELFANTI, P. NICCOLAI, C. PAPUCCI, C. PENTASSUGLIA, M. POGGI, U. SANSONE, E. SCHULTE, G. VENTURA.

I. OBJECTIVES OF THE PROJECT:

Validations of existing theoretical models for representing dispersion and transfer processes in the marine environment and in food chains leading to man. Therefore, a bibliographic study of available models and their potential application to the Mediterranenan will be carried out, combined with field studies in marine and brackish water environments with the objectives to correlate levels of radionuclide concentrations in biotic and abiotic matrices to inputs of radioactivity from different sources and to describe the geobiochemical cycling in the marine environment.

II. OBJECTIVES FOR THE REPORTING PERIOD:

- bibliographic study of available transfer models;
- first field studies in selected brackish and marine sites.

III. PROGRESS ACHIEVED:

Radioecological studies in the Friuli-Venezia Giulia Region

1. Introduction

The radioactive fallout derived from the accident at the Chernobyl nuclear reactor has been used to improve past knowledge of mechanisms of transfer and accumulation of radionuclides in natural and anthropized ecosystems and has provided an unique opportunity for a critical review of environmental transfer models.
The Friuli-Venezia Giulia Region (a region in North East of Italy) was more affected than other Italian regions the radioactive fallout from the Russian nuclear power station and has been chosen as a large and important natural study site in which the principal components of the marine and brackish water environments can be followed for an adequate number of years.
The main aims of the study are as follows:
- Characterization of the environments studies.
- Testing and development of methods for environmental analyses.
- Radiological monitoring.
- Validation of models regarding the dispersion and transfer of radionuclides in the acquatic food chains relevant to man.

First seasonal sampling campaign in the acquatic enviroment

Activities started in spring 1987. Attention was concentrated on the Marano and Grado lagoons and the sea along the caost between Bibione and Duino (Northern part of the Adriatic Sea) because these enviroments are the ultimate recipients of materials eroded by surface run-off and transported by water-bodies. The rivers Stella, Corno, Aussa and Natissa and a number of drainage canals flow general, downstream from the spring-line. The Tagliamento and Isonzo rivers flow directly into the sea, West and East of the Marano and Grado lagoons, and collect most of the water from the mountainous part of the region.
The first seasonal sampling campaign has bee carried out from 22nd June to 4th July 1987 and was aimed at collecting the initial experimental data required to start a complete radioecological characterization research program of the river, lagoon and marine environments for the next 3 years.
These activities have been planned on the basis of the results of environmental studies conducted by the University of Trieste and a National Research Council Institute in the study area. In this way on the basis of these studies and with the direct assistance of the scientists concerned, 54 sampling stations were selected, of which 27 stations on the lagoon, 24 stations at sea, and 3 stations on the rivers.
The following samples have bee collected at these sites:
- marine and lagoon sediments;
- the biologically most important components of the lagoon benthos;
- some species of non-migratory fishes, characteristic of brackish lagoons;

- acquatic plants and algae characteristic of lagoon environments;
- water and suspended material in the lagoon and in the Tagliamento, Isonzo and Stella rivers.

The sampling strategies were defined on the basis of the results obtained from provious investigations and modellization of the study area.

Analyses

Determination of gamma-emitting radionuclides on are in progress. The following additional analyses have also been carried out:

Sediments: Determinations of redox potential, particle size analysis, measurement of total gamma activity at the sediment-water interface.

Biotic components: Taxonomy, determination of fish stomachal contents.

Water: pH, temperature, salinity and dissolved oxygen at various depth.

The definitive programme for the radio ecological study on the lagoon, marine and estuary environments will be defined on the basis of the results obtained in this preliminary stage.

IV. OBJECTIVES FOR THE NEXT REPORING PERIOD:

- application of selected transfer models to Mediterranean coastal environments
- further field studies in selected environments and acquisition of radiological data.

V. OTHER RESEARCH GROUP(S) COLLABORATING ACTIVELY ON THIS PROJECT [NAME(S) AND ADDRESS(ES)]:

Given the multidisciplinary nature of the research project, the necessary skills were found by involving a considerable number of scientist and local government organization and especially:

Local Public Health Board U.S.L. "Triestina" and "Bassa Friulana":
Dr. F. DE GUARRINI
Dr. M. MARINARO
Dr. G. MATASSI

ENEA - COMB:
Dr. R. GIACOMELLI
Dr. M. NOCENTI
Dr. P. SPEZZANO

VI. PUBLICATIONS:

A bibliographic study on the available models and their potential application to the Mediterranean coast has been carried out. On the basis of this study 27 references have been singled out.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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:

Dr.C.N.Chiang

I. Objectives of the project:

The first part of the project was to investigate the possibility of quantitatively predicting the movement of pertechnetate in soils and streams.
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:

Since the part achieved on modeling bacterial growth concluded satisfactorily,the planned work for the present period involves the search for microorganisms able to reduce and/or accumulate Tc-99.
Thanks to facilities available in the project n°2,four lysimeters contaminated in 1984,twice with 0.5 mCi Tc-99 as ammonium pertechnetate per unit,we have started the isolation of microorganisms,bacteria especially in the plough layer,followed by identification of the most representative strains.This should normally gives us a better knowledge of bacteria which have actually grown under contaminated soil.

III. Progress achieved:

1.Methodology:The four lysimeters have been sampled,using PVC pipes of 25 cm lenght with 2.5 cm of diameter.Each pipe,with embedded soil,was sawn in portions of 5 cm,which may be considered as representative layers from 0 to 20 cm of depth.Each layer has been counted for the number of living bacteria,using the classical plate count procedure,to estimate the actual aerobic and anaerobic bacterial population.
Using the dilutions set for the counting,different media were inoculated with soil suspension,namely Plate Count agar,Tryptic Soy,Brain Heart and MRS media.Once the growth was obvious,the most representative colonies were isolated and identified by a procedure developped in this laboratory(Maissin et al.,1987).In addition to these identifications,autoradiographic technique has been used to study their ability to accumulate the Tc-99.The practical steps of which can be outlined as follows:bacteria are grown in media enriched with Tc-99, at a concentration of 10^{-5} M,up to a population density of 10^{7} bacteria/ml,average case being between 24 to 36 hours.An agar film of washed bacteria is further obtained using an haematometer(Thoma cell) and contact exposed with an appropriate film(Hyper film-3H,Amersham code RPN12) during 3 weeks at -40°C.

2.Results:The first observation was the number of the living aerobic bacteria,in terms of average values for the 4 lysimeters(Table I). The populations are relatively small,if compared to what is generally expected under a lawn.The first 5 cm however logically exhibit the highest number and can exceed 10^{7} bacteria/gram of dry soil.For the anaerobic bacteria,the populations never exceed 10^{4} bacteria/gram, which can be considered as small too,specially if one considers the gravimetric moisture content at the sampling period which was relatively high(40%).The stability of the number of anaerobic bacteria may be used as a proof of the non limitation of oxygen diffusion in this sampled layer.
The identification tests show in the aerobic group of bacteria some significant differences between the populations developped at different depths.From the first 5 cm,gram negative rods and cocci were found,with a majority of species belonging to the family of Rhizobiaceae,represented by Agrobacterium tumefaciens and A.radiobacter.
In the second layer,5-10 cm,different Coryneforms species were found, namely Arthrobacter globiformis and A.simplex.Within the third layer, endospore-forming rods were identified as Bacillus polymyxa,and probably B.macerens and B.circulans.In the last layer,15-20 cm,gram negative facultatively anaerobic rods were isolated whose identification must still be confirmed.
For the autoradiographic studies,after processing of the film,we found sensibilized areas on preparations with bacteria grown on Tc-99 enriched media.But,the variations in repeated preparations make the

results still questionnable, in terms of the reliability of the method adapted. Unless, there could be a difference for the same strain to accumulate the Tc-99.

3.Discussion : The numbers of living aerobic and anaerobic bacteria, rather low, remain an observation difficult to explain. The autoradiographic experiment, developped simultaneously, should normally help to select the strains with ability to accumulate the Tc-99, but so far the lack of precision in the results does not allow further interpretation.

Based on the recent work of Henrot, quoted in the project n°2, for a better description of the isotope dynamic in the soil, further identification of anaerobic strains should be carried out. However, in this long term contamined soil, indications of aerobic bacteria possible ability to accumulate the isotope, may represent a field of investigation.

Table I. Populations found in the 4 lysimeters(1)

Depth (cm)	Lys.1		Lys.2		Lys.3		Lys.4		Average	
	AE	AN	AE	AN	AE	AN	AE	AN	AE	AN
0 - 5	12.0	2.9	9.0	5.3	11.0	2.3	8.0	3.8	10.0	3.6
5 -10	2.6	3.4	2.4	3.5	2.1	3.5	1.6	4.2	2.2	3.7
10-15	2.3	4.6	2.2	4.1	1.3	4.3	1.8	2.8	1.9	3.7
15-20	1.6	-	1.6	3.3	1.7	2.3	-	-	1.6	2.8

(1) AE = aerobic bacteria x 10^6/g.
AN = anaerobic bacteria x 10^3/g.

IV. Objectives for the next reporting period:

For the time being,two groups of bacterial strains are actually further investigated for identification,namely 12 aerobic strains and 14 anaerobic ones.
Their ability to accumulate and/or reduce the Tc-99 will be investigated, more specially as far as the anaerobic strains are concerned,by using the autoradiographic procedure as outlined above.
Simultanously,simulation studies using small soil columns will be carried out according to the procedure of Zamani et al.(1985) with the bacterial species which will appear as the most promising in relation with the reduction and/or accumulation of the Tc-99.

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

-Prof.MYTTENAERE C.,Université Catholique de Louvain,Belgium
-Oak Ridge National Laboratory - ORNL:Dr.Auerbach,Hoffman,Blaybock,USA
-Prof.PIERI J.:Université de Nantes,France
-Prof.CREMERS H.,Katoliek Universiteit Leuven,Belgium

VI. Publications:

R.Maissin,A.Bernard,V.Duquenne,S.Baten,G.Gerard et J.Decallonne.
Caractéristiques d'une procédure d'identification informatisée des Lactobacillaceae.
Belgian Journal of Food Chemistry and Biotechnology,42,176-184(1987)

Title of the project no.: 2.

STUDY OF THE BIOCHEMICAL CYCLE TC-99; UNCERTAINTIES ASSOCIATED WITH PREDICTION

Head(s) of project:

Prof. C. MYTTENAERE

Scientific staff:

COGNEAU, M., DEHUT, J.P., DEPRINS, D., FONSNY, K., HENROT, J.,*
SOMBRE, L., VAN LAER, S. VANDECASTEELE, C.M.**

I. Objectives of the project:

The objectives of the project is to reduce the uncertainty which affects the Tc behaviour in a terrestrial environment thanks to a better knowledge of its long term behaviour in soils (lysimeters contaminated in 1983) and its deposition on plant surfaces (interception and ecological half-life-phytotron allowing to study the wet deposition in relation to climatic and ecological conditions).
The present scope is to reduce the existing uncertainties associated with predictions and later to apply the available techniques to radioisotopes which were deposited on the soil and plant surfaces after the Tchernobyl accident.

II. Objectives for the reporting period:

The long term availability of Technetium deposited on soil was simulated using two compartments sub-models which describe the Tc removal from the plough layer and the evolution of the bioavailability of the fraction remaining in the root zone.

The purpose of the work conducted in 1987 was :

- to validate the model describing the ageing phenomena of Tc in soil
- to study the degradation of the bio-incorporated Tc and its availability to plants
- to estimate the interception of the Tc deposited on non grass types of plant in relation to plant growing status.

* Phd thesis in the Ecology Program of the University of Tennessee; Research work in Oak Ridge National Laboratory (TN)
** Scientific collaborator

III. Progress achieved:

1. Validation of the long term behaviour model in soils

The LLN model was validated, introducing the data collected during the period 1983-1987. The following decay model considering two compartments has been validated.

$$Cs = D_1 \times (R \exp(-\lambda_1 t) + (1 - R) \exp(-\lambda_2 t)) + D_2 \times I \times (R \exp(-\lambda_1 (t - T_2)) + (1 - R) \exp(-\lambda_2 (t - T_2))$$

T_2 = 102 days ; 2 contaminations
Cs is the activity present in the soil per unit of area ($Bq.m^{-2}$)
D_1, D_2 is the activity deposited per unit of area
R is the fraction of the deposited activity associated with the first compartment
I is the indicative value corresponding to the contamination (equal to 0 if $t < T_2$ and to 1 if $t > T_2$)

In our experimental conditions, the values of R indicate that more than 70% of the deposited activity was associated to the first compartment (available Tc - $T_{1/2}$ of ± 50 days). The Tc belonging to the second compartment has a very long $T_{1/2}$: ± 30 years. Such a model may be applied for more than two contaminations and it would be interesting to validate it for regular chemical depositions.

2. Technetium speciation in soils (Ageing of technetium)

Analysis of the Tc transfer from soil to plants has shown that the availability of TcO_4^- deposited on soil decreases with time and that the transfer factor decreases from 464 (1984) to 3 (1987). The phenomena has been observed by different scientists and was explained by its reduction and its association with organic matter. The role of soil bacteria in the sorption-desorption processes has been studied recently (1,2).

The reduction with time of the Tf factors may be described using a two compartments equation which parameters value fit very well with the parameters of the same equation applied to the percolates activities for the same period. Such a result confirms that the Tc of the soil water compartment is only available to plants (TF: $T_{1/2}$ first compartment 35 days; second compartment 3 years).

These observations are in complete agreement with the chemical determination (sequential extractions of the Tc of the samples of soil collected in the lysimeters). The result have shown that :

- the total activity decreases with depth (more than 50% of the total activity in the first 5 cm layer)
- The Tc extracted by water (bioavailable Tc) represents only a very low percentage of the total activity (± 3 % of the total Tc)
- the bound Tc is the most important fraction for the different depths (55% bound to organic substrates; 33% bound to sesquioxides; 9% removed by ashing and acid digestion).

3. Final balance of the Tc in the soils

Taking into account of the results obtained in the lysimeters and of the very low bioavailability of residual Tc, a study of the Tc distribution within the three compartments investigated was conducted and the results given by the figure 1. Six years after the first deposition, 27% of the deposited Tc remains in the soil, 41% was exported by the plant and 32% was lixiviated and harvested in the percolates.

4. Availability of the bioincorporated Tc

The figure 2 gives the transfer factors obtained in minilysimeters filled with soils on which leaves of poplar and needles of harches were deposited. Ray-grass was grown on these soils in order to study the biodegradation of the organic material and the Tc availability of bioincorporated Tc.

After the three first harvest it seems clear that the bioavailability of technetium is higher in the "poplar system" and that the biodegradation of that material is quicker.

Transfer factors expressed in (cpm dry weight plant / cpm dry weight soil), were very high in both media and correspond for the first harvest to the non metabolized fraction of Tc stored in the plant material.
The Tf decrease with time as it was observed in the lysimeters. Nevertheless the Tf of the third harvest increase and that result would correspond to a more intense degradation of the poplar material (will be checked up in 1988).

5. Interception of TcO_4 by plant canopies

The interception factors calculated for wheat at different moments of the growth cycle are given by table 1 (Intensity of the rain : 20 mm/h; activity : 1.4 10^6 Bq m^{-2} traced with $Tc\text{-}95_m$).
Values obtained directly after the rain are situated within the range of the values found in the literature for grass (0,02 - 0,82). The highest values obtained for plants harvested at maturity (long-term r values) may be explained by the absorption of Tc deposited on the soil and reabsorbed by the plants. These values would have to be checked up for other non grass types of vegetation.

Table 1

Interception factor

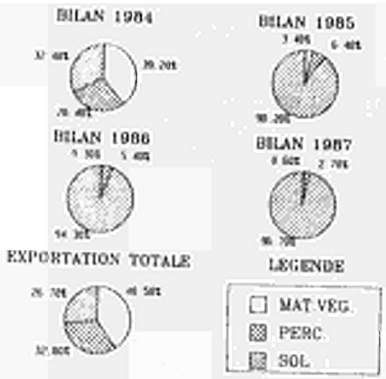
	Short term (1)	Long term (2)
Tillering	0.12	0.68
Flowering	0.18	0.59
Maturity	0.17	0.27
Harvest	0.25	

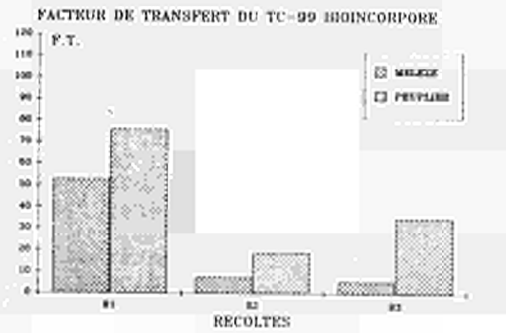
(1) directly after the contamination
(2) at the harvest time

FIGURES

Fig. 1 - Tc distribution within the three compartments (plant, soil, percolates) in relation to time.

Fig. 2 - Transfer factors (cpm dry weight ray grass / cpm dry weight soil) for bioincorporated Tc.





IV. Objectives for the next reporting period:

- Validation of the long term model for Tc-99;
- Study of the transfer of bioincorporated Tc;
- Study of the direct contamination of non grass-type plants by Tc;
- Application of the methods to the Cs-137 contamination of trees (study of the biogeochemical behavior of Cs-137 in forest ecosystems)

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

- Prof. CREMERS: KUL (Katoliek Universiteit Leuven, Belgium)
- Prof. PIERI, J.: Université de Nantes (F).
- Oak Ridge National Laboratory - ORNL: Dr. Auerbach, Hoffman, Blaylock.
- Pacific Northwest Labor. Richland, Washington: Prof. Wildung, Dr. Cataldo.

VI. Publications:

1. HENROT, J. Behavior of technetium in soil: sorption-absorption processes, PHD, University of Tennesse, Knoxville.

2. HENROT, J. Bioaccumulation and chemical modification of technetium by soil bacteria. Report of a Research sponsered by the Office of Health and Environmental Research, US DOE. Contract N° DEAC05-840R21400 with Martin Marietta Energy Systems, Inc.

3. SOMBRE, L. Contribution à l'étude du transfert du radiocésium dans une chaîne d'eau douce simplifiée Eau-Algue verte (Scenedesmus obliquus) - Mollusque filtreur (Dreissena Polymorpha). Thèse doctorat 3ème cycle en Ecologie (Radiohydrobiologie).

4. VANDECASTEELE, C.M. Influence du technetium sur la nitrogénase d'Azotobacter. Thèse de doctorat en Sciences Agronomiques.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Contract no. BI6-B-046-UK

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

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
J Woodyatt

I. Objectives of the project:

The objectives are to examine the influence of geography and climate on the nuclear weapons fallout 'baseline' in atmospheric deposition, in soil and in vegetation, and to apply these 'baseline' data to parallel studies in the environment of nuclear installations. Samples will be analysed for Sr-90, Am-241, plutonium and gamma-emitters including Cs-134 and Cs-137. The continuous measurements will allow seasonal effects to be established and an atmospheric input/vegetation output inventory of radionuclides will be derived at specific sites.

An associated collaborative study is being made in France by CEA Cadarache extending southwards to 44°N (see Section V) for comparison with the measurements in Gt. Britain which extend northwards to 58°N. Some of the study sites are located near to the coast so that the significance of sea to land transfer of actinides can be assessed.

II. Objectives for the reporting period:

(a) to maintain continuous sampling at the 11 field plots of atmospheric deposition, ryegrass and lucerne, including sampling of herbage grown in standard soil with low radionuclide content. The samples from sub-plots indicate the importance of atmospheric deposition of radionuclides by eliminating the effects of soil variability from site to site, and can thus demonstrate the influence of nuclear installations.
(b) to complete analysis of samples collected in 1986 and to commence analysis of 1987 samples for gamma-emitters and actinides, and stable Na and Ti to assess deposition of sea spray and soil dust. Particle size distributions in rainwater will be measured.
(c) to sample soil profiles at each site and to commence analysis of radionuclides to establish the extent of migration.
(d) to collaborate with CEA Cadarache on implementation of the project, and comparison of results.

III. Progress achieved:

The routine collection of total (wet and dry) deposition, ryegrass and lucerne has proceeded at the 11 field plots (Fig. 1). The vegetation is now well established and receives standard fertiliser and herbicide treatments: it was harvested at the end of May 1987 and again in September. Soil profiles have been sampled to 0.5 m depth from grassland next to the plots, by removing soil from 12 depth intervals.

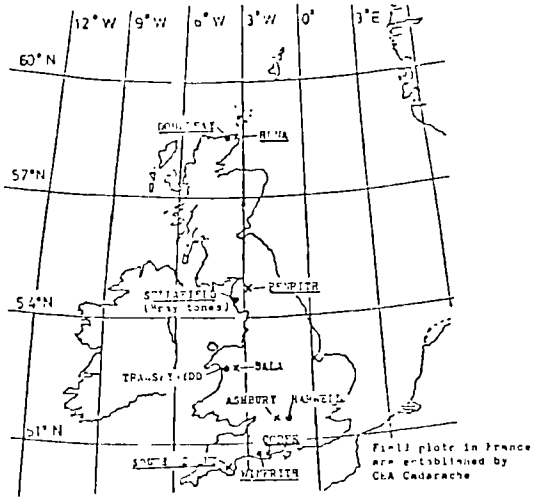


FIGURE 1 LOCATION OF FIELD PLOTS FOR MEASUREMENT OF MAN MADE NUCLIDES

Concentrations of Cs-134 and 137 in ryegrass had decreased by 2 orders of magnitude at 5 months after the Chernobyl accident, and did not exceed 100 Bq Cs-137/kg dry weight; lucerne shows much less uptake. The influence of nuclear installations on environmental levels of radionuclides is evident, by order of magnitude increases in soil concentrations of plutonium and Am-241 at the Dounreay and Braystones (near Sellafield) plots, with a similar increase in the seasonal atmospheric deposition of actinides and concentrations in ryegrass and lucerne, ie, up to 0.7 Bq Pu-239+240 kg^{-1} and 0.9 Bq Am-241 kg^{-1}. Results from sub-plots indicate that these increases occur by interception of actinides by foliage and that soil to plant transfer is relatively minor.

Examination of particulate matter in deposition and on leaves shows three main groups, namely spherical (1 to 10 μm dia.), angular (5 to 15 μm) and aggregate (16 to 50 μm). Up to 25 x 10^6 particles/ litre are present, but the association with radionuclides is unknown.

IV. Objectives for the next reporting period:

(a) to maintain the sampling programme for atmospheric deposition and vegetation according to the established schedule and to continue with the analysis of gamma-emitters and actinides in all samples. 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.

(c) to use the data from plots that are distant from nuclear installations to assess the climatic effect on radionuclide deposition and accumulation in soil.

(d) 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, 13115 Saint-Paul-lez-Durance, France.

Collaboration is made with A. Grauby, J. Delmas and C. Colle. Discussions took place with CEA Cadarache in autumn 1987 and results were discussed at the 5th Workgroup Meeting on Soil to Plant Transfer at Egham GB, April 1987.

VI. Publications:

R S Cambray, P Burton, P A Cawse et al. (1987). Observations on radioactivity from the Chernobyl accident. Nuclear Energy, 26: 77-101.

Short Communication/Summary

P A Cawse and S J Baker (1987). Atmospheric deposition of radionuclides and transfer to vegetation in Gt. Britain, 49-50, In 5th Report of Workgroup on Soil to Plant Transfer Factors, Egham, UK, April 1987, RIVM BILTHOVEN, The Netherlands.

Title of the 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
D Jenkins

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 Am-241 and Np-237 tracer experiments in 1986, and to continue the harvesting programme throughout 1987. 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 which is intended to proceed for three years, using ryegrass and lettuce as test plants.

(b) to set up a further tracer experiment using Cm-244 in nitrate form added to an organic (fen) soil and a brown earth.

(c) to collaborate with CEA Cadarache, who are carrying out a series of experiments with additional crop plants using the same organic (fen) soil from Gt. Britain, and French soils (brown acidic and brown calcareous earth).

III. Progress achieved:

Analysis of samples collected in Autumn 1986 and Spring 1987 has proceeded and soil to plant transfer factors for Np-237 and Am-241 have been derived. In Summer 1987, further samples of ryegrass and lettuce crops have been obtained and prepared for analysis.

The results from the first year of ryegrass growth show that according to the soil type and treatment, the concentrations of Np-237 and Am-241 vary from 0.8 to 95 Bq kg^{-1} and 0.7 to 100 Bq kg^{-1} dry wt. respectively. Thus the soil to plant transfer factors for ryegrass were in the following ranges:

Np-237 1.1×10^{-4} to 0.13
Am-241 3.9×10^{-5} to 5.4×10^{-3}

The highest transfer factors for both radionuclides occurred with cultivation in brown earth (sol acide) and also in sterilised organic soil for Am-241, where uptake was increased by an order of magnitude. The application of sewage sludge to ryegrass was found to increase transfer factors and the total removal of both Np-237 and Am-241. Up to the present, uptake of the tracers by ryegrass in the combined (synergistic) shows similar transfer factors to the single treatment.

Lettuce from the organic soil contained 26 Bq Np-237 kg^{-1} compared with 30 Bq kg^{-1} in ryegrass during the same (Autumn) growth period.

In July 1987, 5 plastic containers each holding 15 kg (dry weight) of soil received Cm-244 tracer in nitrate form, mixed into the soil layer by layer to achieve a soil concentration of 0.5 μCi (18.5 kBq) kg^{-1} dry soil. The treatments were:-

Organic (fen) soil with ryegrass as test plant	Organic (fen) soil with lettuce as test plant
+ Cm	+ Cm
+ Cm and gamma-sterilised	
+ Cm and sewage sludge	

Brown earth (from Sellafield) with ryegrass as test plant

+ Cm

The soil sterilisation was carried out by treatment with 5 Megarads of gamma-radiation in a commercial irradiation facility. The first harvest of the test plants was made in late Autumn 1987: analysis is carried out on 'Winter' and 'Summer' vegetation, following the scheme used for the other tracer experiments.

IV. Objectives for the next reporting period:

Analysis of vegetation samples collected in 1987 will be completed for comparison with results from the first year. Further samples of ryegrass and lettuce will be grown in 1988, the lettuce being re-sown in Spring. Soil samples will be taken at the end of the growing season to assess the distribution of Np-237 and Am-241 in soil fractions. The tracer study using Cm-244 will be carried out with ryegrass and lettuce according to the schedule used for the other tracers. The results will be discussed with CEA Cadarache.

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, 113115 Saint-Paul-lez Durance, France.

Collaboration is made with A. Grauby, J. Delmas and C. Colle. Some of the crop samples obtained by Harwell have been analysed for Np-237 and Am-241 by CEA, for comparison purposes.

VI. Publications:

(Experimental work still in progress).

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

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:

(1) Sampling on the Irish Sea coast to be continued using size selective samplers, to size and collect the aerosol between about 0.2 and 10 μm diameter, and optical methods to measure the size distribution of the total aerosol in situe.

(2) The field bubbling system to be adapted for use in a laboratory seawater tank. A laser diffraction technique will be used to size the bubbles and, if possible, the aerosol generated.

III Progress achieved

(1) Field Programme

Techniques to measure both the distribution of droplet sizes and the relative concentration of the droplets within the marine aerosol blown ashore have been used on the Cumbrian coast. Some development and calibration is still required but results are very promising. The apparatus is set up a few metres above the highwater mark. At this location measurements have demonstrated marine aerosol particles from 2.5 to 90 μm diameter.

(2) Laboratory Programme

The field bubbling system has been successfully adapted for use in the laboratory. Some preliminary experiments on the influence of seawater organic/surfactant content on bubble size and aerosol production and have shown a substantial effect of natural and artificial organic material on aerosol generation rate and bubble size distribution.

IV. Objectives for the next reporting period:

Gain a greater understanding of (1) the importance of seawater dissolved organic content on aerosol flux and sediment loading on aerosol particulate content, and thus actinide sea to air transfer (2) the variation in beach aerosol size distribution and concentration with wind speed and distance from the surf zone. Objective 1 will be carried out in collaboration with the University of East Anglia.

V. Other research group(s) collaborating 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 Yves Belot
Commissariat a l'Energie Atomique
Department d'Etudes et de Recherches
en Securite
Boite Postale 6
92260 Fontenay-aux-Roses
France

VI. Publications:

RADIATION PROTECTION PROGRAMME

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1987

Contractor: Contract no.: BI6-B-191-NL

Delta Institute for
Hydrobiological Research
Vierstraat 28
NL-4401 EA Yerseke

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

Dr. E.K. Duursma
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: Dr. E.K. Duursma, Dr. D. Eisma. Nederlands Instituut voor Onderzoek der Zee (Texel, The Netherlands)

Dr. J.M. Martin. Institut de Biogéochimie Marine (E.N.S. France)

Dr. J. Pentreath. Fisheries Radiological Laboratory (Lowestoft, England)

Dr. Prof. R. Wollast. Université Libre de Bruxelles (Belgique)

Scientific staff:
Dr. D. Petit. Institut de Biogéochimie Marine (E.N.S. France)
J. Nieuwenhuize. Delta Instituut voor Hydrobiologisch Onderzoek (Yerseke, The Netherlands)

I. Objectives of the project:

Study of total dissolved and particulate Pu-238 and Pu-239, 240 and γ emitters (Co-60, Cs-137,...) in the Western Scheldt area.
Determination of Kd's as function of major physicochemical 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.

II. Objectives for the reporting period:

Sampling and analytical methods for measuring total Pu (suspended matters and waters) and redox Pu speciation(waters) have been tested in estuarine waters. The samples have been collected along the Western Scheldt estuary where specific sources additional to fallout Pu have been measured in suspended matter and sediments during a preceding cruise.

III. Progress achieved:

1. Methodology

Surface waters (about 200 l) and suspended matters from the Western Scheldt were collected in June, August and October 1987 on board R/V Luctor (DIHO, Yerseke, The Netherlands).
The samples collected during the first and the second cruises came from three monitoring stations located along the Western Scheldt (Rupelmonde, Doel and Schaar Van Spijkerplaat).
Suspended matter was recovered on the spot by continuous centrifugation. Dissolved water was obtained by pressure filtration on 0.4 um membrane filters. Recoveries were determined by the addition of yield monitors (Pu-242 or Pu-236). The samples were chemically separated and analyzed for total Pu according to the method given by Heterington (1976). Large volume samples (about 350 l) were collected in August 1987 for Pu redox speciation. These samples filtered on 0.4 um filter porosity and spiked with Pu-242 (III, IV) and Pu-236 (V, VI) were analyzed following the Fe $(OH)_3$ coprecipitation method given by Penthreath et al. (1985).

2. Results and discussion

2.1 Total Pu activities

The Pu activities of filtered surface waters and suspended matters from the December 1986, June and August 1987, respectively are given Table 1. As seen from these data, total dissolved Pu measured in June and August 1987 at the estuarine stations (Rupelmonde and Doel) were very low as compared with the samples taken at the same stations in December 1986.
Although the geochemistry of Pu in estuarine waters is not well documented, a recent paper of Sholkovitz et al. (1987), which has measured the activities of dissolved Pu in four estuaries on the north-east coast of the United States found very low Pu values in waters collected in the zone of turbidity maximum where salinities range from 0 to about 10 ‰. These dissolved Pu activities are very similar to our June and August 1987 cruises results.
The higher dissolved Pu values found in our estuarine stations during December 1986 cruise might be due to some artifacts like (1) increased fallout from Chernobyl accident (2) Pu contamination in the laboratory during chemistry samples processing or (3) change of filter pore size for iron hydroxydes recovery (in December 1986, we utilized 0,2u porosity instead of 0,4µ which were normally used for all other cruises). Low Pu laboratory blanks measured during December sample analyses and Pu-238/Pu-239 activity ratios found in dissolved samples, which were very different from Chernobyl one's (Pu-238/Pu-239 = 0.4 - 0.5), excluded the first and the second hypothesis.

On the contrary, the last one cannot be ruled out. Previous studies have shown that "dissolved Pu" in estuaries might be removed from the dissolved phase by coagulation of colloïdal particles originating from freshwater. As an example Shen et al. (1983) have showed, that the major part of "dissolved Pu" exists as colloïds in the organic rich fresh-water end member of estuaries.
Particulate Pu did not follow the same trend and show activities which are almost constant for all the cruises (see Table 1.).

2.2 Pu redox speciation

Analyses of samples collected in August 1987 showed that, contrary to the first redox speciation experiment, made in December 1986, the separation of the reduced Pu-242 and the oxidized Pu-236 spikes were quite adequate. The degree of cross contamination was of the same order of magnitude (10 - 15 %) than the Lowestoft laboratory results found for marine waters contaminated by waste Pu effluents from Sellafield (Harvey and Lovett, 1984). Unfortunately , because of the very low activities found in the estuarine water samples (see Table 1) it has not been possible to measure Pu redox speciation in these samples.

Station	Date of sampling	Waters Pu-239-40 (fci/l)	Waters Pu-238/Pu-239	Suspended matters Pu-239-40 (fci/g)	Suspended matters Pu-238/Pu-239
R*	December 1986	0.124±0.013	0.30±0.05	17.6±1.0	0.45±0.05
D*	"	0.098±0.009	0.27±0.05	19.4±0.8	0.35±0.03
S*	"	0.110±0.013	0.24±0.06	47.7±1.1	0.31±0.01
R	June 1987	0.005±0.002	-	15.2±0.4	0.40±0.04
D	"	0.007±0.001	-	12.0±0.9	0.37±0.05
S	"	0.081±0.005	0.39±0.04	41.9±1.6	0.29±0.03
R	August 1987	0.003±0.001	-	16.2±0.6	0.42±0.02
D	"	0.008±0.002	0.21±0.10	15.0±0.8	0.35±0.02
S	"	0.048±0.004	0.29±0.06	35.5±0.9	0.30±0.04

R*: Rupelmonde ; D*: Doel ; S*: Schaar Van Spijkerplaat.

Table 1. Results from the Pu 239-240 and Pu-238/Pu-239 activity ratio analysis in the water and suspended matters of the Scheldt estuary (errors are expressed as 1 standard deviation).

References

Harvey B.R. and Lovett M.B. (1984). Nucl. Instrum. and Meth. in Phys. Res., 223, 224-234.

Heterington J.A. (1976). Environmental toxicity of aquatic radionuclides: models and mechanisms, Miller M.W. and Standard J.N., Eds, Ann. Arbor Sci. Publ. Inc. Michigan, 81-106.

Lovett M.B. and Nelson D.M. (1981). Techniques for Identifying Transuranic Speciation in Aquatic Environments, 24-28 March 1980, Ispra, Italy, IAEA 27-35.

Pentreath R.J., Harvey B.R. and Lovett M.B. (1985). Speciation of Fission and Activation Products in the Environment, Bulman R.A. and Cooper J.R. Eds, Elsevier, London, 312-325.

Shen G.T., Sholkovitz E.R. and Mann D.R. (1983). Earth Planet. Sci. lett., 64, 437-444.

Sholkovitz E.R. and Mann D.R. (1987). Estuarine, Coastal and Shelf Science, 25, 413-434.

IV. Objectives for the next reporting period:

In order to locate more precisely the sources of Pu present in the Scheldt estuary, we have decided to analyse samples collected by centrifugation or sediment traps since July 1986 by the Brussels laboratory (ULB) in the Scheldt (Antwerp Hemiksem, Doel, Gent) and in some of its tributaries (Rupel, Dyle, Nethe). For the last cruise we project to complete our investigation of the Pu sources found in the Scheldt estuary by sampling waters and suspended matter samples from the Scheldt above the tidal intrusion at Gent and in various Scheldt tributaries. We also plan to conduct laboratory adsorption experiments made with Pu-237 ($T_{\frac{1}{2}}$ = 42 d., γ emitter) on small estuarine waters volume (about 1 l) to test the stability of the reduced (III, IV) and oxidized (V, VI) forms of Pu in oxic and anoxic conditions.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

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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 indentify 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 of analysis radionuclide in the megafauna collected at the site will be carried out.

II. Objectives for the reporting period: 1 dec. - 31 dec. 1987

- Starting the project
- Organize the expeditions
- Contact with benthic taxonomists

III. Progress achieved:

As the project has just been started (1 dec. 1987) the planning for 1988 will be given here.

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 will be collected. Furthermore, a photocamera will be used to take photographs of the epibenthic community.

Results and discussion:

Results on the following 4 topics will be aimed for:

Biology 1) Comparison of the meio- and macrobenthic community of the NEA-dumpsite with other nearby deep sea areas, especially those situated downstream. Because of the DORA-project (1982-1986), also partly subsidized by the Commission of the European Communities (Contract no. B16-054-NL) the dumpsite is one of the best known deep-sea areas with respect to the benthos. However, the surrounding-areas are poorly known, and as radionuclides can eventually reach these areas, it is necessary to study the difference between these areas and the dumpsite.

2) A further study of the deep sea foodweb. During the DORA-project research on the trophic levels within the Nematoda and on the diet of bentho-pelagic fishes has been carried out. The present research will give more in-depth knowledge including more animal groups. In practice this will mean:

a) Splitting up the benthic groups in smaller taxa to enable the dividing into different trophic levels.
b) Visual stomach content research of megafauna e.g. anemones, starfishes, seasquirts and fishes.

3) Quantitative measurements of megafauna. To complete our knowledge of the ecosystems of the site, the density and biomass of the megafauna, of which almost no data are known will be obtained with the use of a bottom trawl and a deep sea camera.

Radionuclide analysis

4) To verify if there is an actual transport of radionuclides from the waste canisters to benthic organisms, several megafauna animals will be analised for α and γ emitting radionuclides.

IV Objectives for the next reporting period:

- Collection of biological samples (meio-, macro- and megafauna) during expeditions with the FS. Meteor (march-april) and MS. Tyro (june-july) at or near the NEA-dumpsite.
- Starting the biological research on the distribution of the benthos, the comparison of the deep sea fauna and the study of the foodweb.
- Radionuclide analyses of megafauna.

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).

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
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1987

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.Stoutjesdijk and J.F.Lembrechts

Scientific staff:

G.M.Desmet, M.J.Frissel, H.W.Köster, J.F.Lembrechts and J.F.Stoutjesdijk

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 effect and persistence of a once-only applied countermeasure on the bioavailability of radionuclides for crops will change in the course of time, just as availability as such. A repeated application might be required to induce a perceptible effect or to enhance or consolidate it. Therefore, the production plan and countermeasures of 1986 were in part repeated in 1987.

- Pot experiments were carried out to investigate the bioavailability of Sr-85 and Co-57 in relation to soil solution chemistry.

III. Progress achieved:

METHODOLOGY

1. Experiments under natural conditions have been carried out on the fields of the lysimeter, described in earlier reports. In February all containers were recontaminated with Sr-85. The countermeasures studied last year were repeated, with the exception of addition of stable Zn and Mn; the various treatments being: omission of normal fertilization, addition of organic matter (455,000 kg/ha for löss and clay, and 364,000 kg/ha for sand) and of lime (2250 kg $Ca(OH)_2$/ha). Beans and spinach were grown successively, on all containers. The radionuclide content of plant and soil were measured and in June and September some chemical characteristics of the interstitial soil liquid phase (pH, conductivity, total γ-activity) were analysed.

2. The relation between uptake of Sr-85 and soil solution chemistry was studied using lettuce, grown for 2 to 5 weeks on a löss or sandy soil (about 1 kg/pot). Soil pH and conductivity, and radionuclide content of plant and soil solution were measured.

3. The effect of different levels of fertilization, of addition of organic matter or stable Co and of liming following acidification upon the presence of Co-57 in the soil liquid phase of a sandy soil (100 Bq/kg) were studied.

RESULTS AND DISCUSSION

1A. Soil liquid phase - The Mg- and K-concentration of the soil solution clearly decreased in unfertilized soils (x 0.5), without, however, affecting yields. Application of organic matter mainly affected the moisture content (x 1.5 to 2.0), the K-concentration and the yield of beans (x 2 to 5). Liming increased the Ca-concentration (x 1.5) and pH (from 5.5 to 6.5) of the liquid phase of the sandy soil. Solely in the soil solution of a sandy soil the addition of stable Zn and Mn of 1986 remained visible ([Zn] x 3).

1B. Transfer factors (TF) - An elaborated statistical analysis remains to be done. At a glance, however, the uptake of all elements analysed by both plants and from all soils appeared to decrease significantly and almost systematically (table 1). This year one could observe a difference comparable to that of 1986 in the availability of both isotopes of Cs, which were added at different times.

1C. Countermeasures - Although being pronouncedly applied all countermeasures seemed to have a fractional effect as compared to the changes observed between the two succeeding growing seasons.

2. In an attempt to relate uptake of Sr-85 to the composition of the soil solution the effect of an NPK-fertilizer (0.25 to 0.50 g/kg Sporumix PG) and of $Ca(OH)_2$ (1 or 2 g/kg) was studied. The quantity of freely dissolved Sr-85 increased directly proportional to the conductivity of the liquid phase ($r = 0.995$, $N = 28$). This relation did not depend on the soil matrix and was not influenced by the presence of plants or by the growth period. The increased availability upon administration of fertilizer was, however, counterbalanced by an inhibiting effect of the fertilizer on absorption of Sr-85. The transfer expressed on the basis of the soil solution activity thus decreases as a function of conductivity (fig. 1), whereas that based on the total activity remains constant

Comparable effects have been described for Tc (previous progress report).

After 3 months of incubation the concentration of freely dissolved Co-57 was not affected by an addition of organic matter (up to 6.9%), only changed after a five- to tenfold supply of stable Co (8 to 15 mg/kg), and increased linearly with the supply of fertilizer (0.05 to 0.5 g/kg Sporumix), whereas dissolution of Co-57 induced upon acidification (down to pH 4) could not be completely reversed by an addition of $Ca(OH)_2$.

TABLE 1. TF in 1987 / TF 1986 (mean ± standard error)

	Co-57	Co-60	Cs-137	Cs-134	Mn-54	Zn-65
Spinach:						
löss	0.33 ± 0.18	0.33 ± 0.21	0.30 ± 0.24	0.22 ± 0.17	0.37 ± 0.13	0.72 ± 0.10
clay	0.17 ± 0.10	0.22 ± 0.19	0.20 ± 0.17	0.14 ± 0.10	0.27 ± 0.15	0.93 ± 0.83
sand	0.57 ± 0.15	0.46 ± 0.09	0.26 ± 0.06	0.31 ± 0.05	0.47 ± 0.20	1.26 ± 0.36
Beans:						
löss	0.50 ± 0.12	0.55 ± 0.14	0.29 ± 0.09	0.28 ± 0.09	0.69 ± 0.27	0.67 ± 0.20
clay	0.33 ± 0.05	0.32 ± 0.05	0.14 ± 0.03	0.11 ± 0.03	0.34 ± 0.12	0.61 ± 0.24
sand	0.84 ± 0.41	0.94 ± 0.42	0.94 ± 0.33	0.91 ± 0.36	1.05 ± 0.78	1.63 ± 0.52

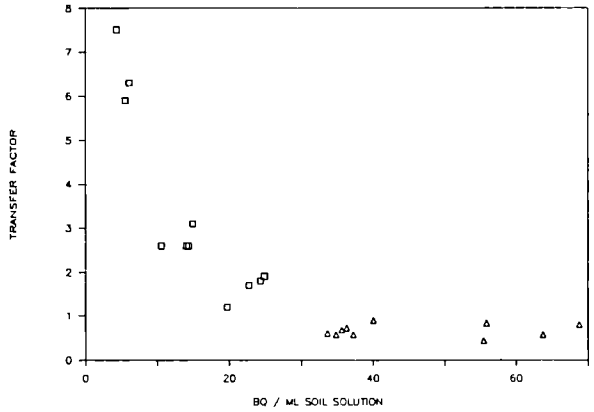

FIG.1. Transfer of Sr-85 (Bq/g/Bq/ml) from soil (löss: □ and sand: Δ) to plant (Lactura sativa L.) as a function of soil solution concentration.

IV. Objectives for the next reporting period:

a. A further analysis of the impact of the selected countermeasures (liming, addition of organic matter and stable isotopes, and omission of fertilizer) on transfer of radionuclides from soil to crop. Assessment of the variability and of the persistence of effects.

b. Generalization of the experimental techniques and of the model developed to study the accumulation of Tc in relation to its bioavailability. This mainly includes the assessment of availability by means of the soil solution displacement method.

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

Prof.Dr.Ir.A.Cremers, K.U.L., Leuven, Belgium
Prof.Dr.O.Vanderborght, U.I.A., Antwerpen, Belgium

VI. Publications:

Frissel, M.J. and Köster, H.W.: Soil-to-plant transfer factors of radionuclides. Expected values and uncertainties. A summary of available data. Proc. of the Vth Workshop of the I.U.R., December 1987, 2-25

Stoutjesdijk, J.F., van Ginkel, J.H. and Pennders, R.M.J.: Determination of soil-plant transfer factors and measures to influence the uptake of radionuclides. Proc. of the Vth Workshop of the IUR, December 1987, 26-37

Frissel, M.J., Stoutjesdijk, J.F., Koolwijk, A.C. and Köster, H.W.: The Cs-137 contamination of soils in the Netherlands and its consequences for the contamination of crop products. Netherlands Journal of Agricultural Science 35 (1987) 339-346

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Kernforschungsanlage Jülich
Postfach 1913
D-5170 Jülich

Contract no.: BI6-B-053-D

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

Prof. Dr. F. Führ
Institut für Radioagronomie
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Telephone number: 02461/61.63.92

Title of the research contract

Simulation of transfer via the soil-plant food chain after accidental release.

List of projects:

1. Simulation of transfer via the soil-plant food chain after accidental release.

Title of the project no.: BI 6-053-D

The soil-plant transfer of the radionuclides Cs-137/134 and Co-60/57 after simulation of a deposition subsequent to a long-term and accidental release from nuclear plants.

Head(s) of project: Dr. W. Steffens

Scientific staff: Dipl. Geogr. M. Bilo, Prof. Dr. F. Führ, J. Klaes, Dipl. Ing. W. Mittelstaedt, Dr. W. Steffens

I. Objectives of the project:

1. Determination of transfer factors describing the uptake of the radionuclides Cs-137/134 and Co-60/57 from soil into plants and food chains after simulation of a long-term contamination of the soil and of a high deposition of these radionuclides on soil and plants subsequent to an accidental release.
2. Investigation of the effects of different element concentrations in the soil on the transfer of Cs-137/134 and Co-60/57.
3. Effect and applicability of various soil tillage methods (normal or deep ploughing) to reduce the soil-plant tranfer of the radionuclides.
4. Comparison of the transfer factors determined in Cadarache/France and Jülich/F.R.G. with regard to the differing climatic conditions.

II. Objectives for the reporting period:

1. Transfer of Cs-137/134 and Co-60/57 from soil into carrot leaves and roots, calculation of transfer factors dependent on the element concentration and the duration of these radionuclides in the soil.
2. Applicability of normal ploughing and deep placement of the top 6 cm soil layer into 34-40 cm depth to reduce the root uptake of Cs-137/134 and Co-60/57 and the contamination of the food chain.

III. Progress achieved:

1. Methodology

The first part of these lysimeter experiments, which was related to the transfer of Cs-137 and Co-60 from soil into plants after long-term contamination and to the effects of various Cs- and Co-concentrations on the transfer is described in the Progress Reports 1984 and 1985. In 1986, a high deposition of Cs-134 and Co-57 subsequent to an accidental release from a nuclear installation was simulated by spraying an aqueous solution containing these radionuclides and various Cs- and Co-concentrations either on soil or plant surfaces. The element concentrations are shwon in table 1.

In 1987, the soil of 8 lysimeters (even numbers) was tilled 30 cm deep. Thus the Cs-134 and Co-57 applied onto the soil surface in 1986 was distributed in the ploughed soil layer. The top 6 cm layer of the other groups with 8 lysimeters (odd numbers) were taken off and after removal of the next 34 cm soil layer placed into a depth of 34-40 cm and covered with the layer removed before. The radioactivity determined in the soil is shown in table 2 accept for the lysimeters with deep placement of the 0-6 cm layer containing Cs-134 and Co-57. In these lysimeters the total radioactivity (mean values/lysimeter) determined in the soil amounted to about 22.6 and 25.0 MBq for Cs-134 and 29.3 and 28 MBq for Co-57, respectively.

In all lysimeters carrots were grown. The radioactivity in the plants was determined and the data were used to calculate transfer factors.

Table 1: Element concentration of Cs and Co in the soil after repeated application (1984 and 1986)
Natural concentration in the soil: Cs = 1.4 mg/kg, Co = 5.8 mg/kg

	Element concentration, mg/kg of soil Mean values of 2 lysimeters each, 0-30 cm layer							
	1+3	2+4	5+7	6+8	9+11	10+12	13+15	14+16
Cs	1.4	1.4	1.4	6.7	7.9	10.6	7.9	15.9
Co	49.6	74.6	49.6	67.8	5.8	19.4	5.8	5.8

Table 2: Radioactivity of Cs-137/134 and Co-60/57 determined in the soil, 0-30 cm layer

Lysimeter Nr.	Mean Radioactivity, Bq/g of dry soil			
	Cs-137	Cs-134	Co-60	Co-57
1, 3, 5, 7	100		95	
2, 4, 6, 8,	140	284	133	355
9, 11, 13, 15	97		107	
10, 12, 14, 16	134	351	140	406

2. Results

The results in table 3 and 4 are mean values from 2 lysimeters each. The transfer factors for Cs-137/134 and Co-60/57 calculated in carrot roots increased with increasing concentration of Cs- and Co in the soil. For Cs-134 and Co-57 distinctly higher transfer factors were determined (table 3). Deep placement of Cs-134 and Co-57 had no reducing effect on the uptake of these radionuclides into carrot roots. But in the case of high concentration of Cs and Co in the soil the uptake of Cs-134 and Co-57 was increased after deep placement (table 4).

3. Discussion

The results obtained for Cs-137 and Co-60 confirm the transfer data of the 3 previous experimental periods indicating, that additional Cs and Co disturbes the equilibrium in the soil and increases the availability of these radionuclides for root uptake. The differences between the transfer factors of the 2 Cs- and Co-radionuklides might be due to the different time of application onto the soil. The availability of Cs-134 and Co-57 is still higher, because these radionuklicdes were applied 2 years later than Cs-137 and Co-60. These results demonstrate, that a longer time period is needed to reach an equilibrium stage in the soil. For carrots a placement of radionuclides into deeper soil zones seems to have no effect, supposingly due to the fact that carrot roots are penetrating relatively deep into the soil.

Table 3: Transfer factors for Cs-137/134 and Co-60/57 in carrot roots

Lysimeter Nr.	Mean Transfer factors (F.W.)			
	Cs-137	Cs-134	Co-60	Co-57
1 + 3	4.49 E-4		3.07 E-3	
2 + 4	5.15 E-4	1.48 E-3	2.90 E-3	5.47 E-3
5 + 7	3.37 E-3		3.22 E-3	
6 + 8	2.33 E-3	1.09 E-2	1.97 E-3	5.70 E-3
9 + 11	3.26 E-3		2.45 E-3	
10 + 12	3.91 E-3	7.42 E-3	1.18 E-3	2.15 E-3
13 + 15	5.67 E-3		9.30 E-4	
14 + 16	7.14 E-3	2.95 E-2	8.88 E-4	1.09 E-3

Table 4: Radioactivity in carrot roots after distribution of Cs-134 and Co-57 in the 0-30 cm soil layer and after deep placement (34-40 cm).
Bq/g plant dry matter related to 10 MBq of each radionuclide in the soil.

Element concentration in the soil	Radioactivity, Bq/g P.D.M.			
	Cs-134		Co-57	
	Distribution in 0-30 cm	Deep placement	Distribution in 0-30 cm	Deep placement
Low	1.0	0.9	0.7	0.5
High	19.2	35.7	3.8	9.0

IV. Objectives for the next reporting period:

1. Continuation of the experiments on the transfer of Cs-137/134 and Co-60/57 from soil into plants dependent on the element concentration in the soil and the kind of radionuclide deposition - long-term low contamination and high accidental deposition.
2. Investigation on the reducing effect of tillage and deep placement on the transfer of Cs-137/134 and Co-60/57 after deposition onto the soil surface.

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

Institute de Protection et de Suretée Nucléaire (IPSN) Départment d'Etude et des Recherches sur l'Environment (SERE), CEN Cadarache, 13115 Saint Paul Lez Durance, France.

VI. Publications:

none

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Kernforschungsanlage Jülich
Postfach 1913
D-5170 Jülich

Contract no.: B16-B-189-D

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

Prof. Dr. F. Führ
Institut für Radioagronomie
KFA Jülich GmbH
Postfach 1913
D-5170 Jülich

Telephone number: 02461/616392

Title of the research contract:

Conversion of elementary tritium (HT) in agriculturally used soils, oxidation of HT to HTO and synthesis to organically bound tritium.

List of projects:

1. Conversion of elementary tritium (HT) in agriculturally used soils, oxidation of HT to HTO and synthesis to organically bound tritium.

Title of the project no.:

Conversion of elementary tritium (HT) in agriculturally used soils, oxidation of HT to HTO and synthesis to organically bound tritium (HTO)

Head(s) of project:
Prof. Dr. Führ

Nuclear Research Centre Jülich (KFA), Radioagronomy,
POB 1913, D 5170 Jülich, Fed. Rep. Germany

Scientific staff:
Dr. H. Förstel
Nuclear Research Centre Jülich (KFA), Radioagronomy,
POB 1913, D 5170 Jülich, Fed. Rep. Germany
phone: 02461/ 61 3241

I. Objectives of the project:

The conversion of HT to HTO (elementary tritium to tritiated water) is studied in non-disturbed soil cores taken from the field. The deposition velocity of HT is determined to demontrate its dependence on physical (soil water content, porous space), biological (soil biomass, plant species nitrogen fixation) and chemical (cultivation, fertilisation organic matter content) parameters. The reemission of HTO back to the air and the uptake of tritium into the OBT is observed. Test of the applicability of the method proposed under field conditions (release experiment).

II. Objectives for the reporting period:

According to the importance of the release experiments the samples from both the French and Canadian experiment had priority over the other parts of the programme. The final version of the report about the French release is available. The publication of the results from the Canadian experiment is in preparation. Both releases had confirmed that the laboratory data can be used to sufficiently predict the deposition velocity in the field . A new radiological pathway(soil/air/leaf water) must be taken into account. Laboratory experiments have demonstrated that an increase of the wind velocity results in a growth of the reemission rate. First data of reemission in the field are available. Measurements of the deposition velocity of soil from field, pasture and forest soil at Jülich location were comparable to the results of former years. Stimulation of synthesis of soil biomass results in an increased but nevertheless limited uptake of tritium into OBT (soil incubated with HTO).

III. Progress achieved:

1. Methods

The release experiments generally had to demontrate the importance of bioactive soil as a sink of elementary tritium HT. In the soil the enzymes of microorganisms convert HT into tritiated water HTO as the first reaction product, which is more radiotoxic by a factor 10^4 than HT. For some years we have tested and applied a method which enables us to measure the deposition velocity in natural soils. Undisturbed soil cores are directly taken in the field. In the laboratory their surfaces are exposed to an air stream containing HT. The technical arrangement enables one to measure both the deposition velocity (ionisation chamber) and the reemission rate (cooling traps).

The French release experiment was made in an area predominantly in agricultural use. Two sets of measurements were performed. Firstly, using cores of different soils were collected in the vicinity of the KFA Jülich , the deposition velocities of some representative samples were measured and finally the remaining samples were exposed to the HT plume in the field at point A(1) where most of the other research groups had placed their main equipment. Secondly soil cores were collected in the French release area during a period of two month before and some days directly after the HT release.

The Canadian release experiment had taken place at a forest clearing site from a hight of 1 meter during 30 minutes. The tritium plume and the uptake by the ecosystem was observed downwind up to 400 meters and some distance into the adjacent forest. The deposition velocities of bare and plant covered soil as well as the influence of different plant species were studied. The movement of the reaction product HTO within the soil and the reemission into the air were followed. A double labelling technique using $H_2{}^{18}O$ and HTO simultaneously was applied. A set of soil samples was exposed to the plume to observe its HTO reemission afterwards. HTO can be extracted by azeotropic distillation.

To study the reemission under well-defined laboratory conditions, cores of forest soil were labelled quickly after exposure of their surface to HT. Thereafter, a well-defined flow of humid air is led over the soil surface. The reemitted HTO is trapped by a set of water flasks.

During uptake of HT by the soil no organically bound tritium is formed initially, only a small fraction of HTO is taken up into the biomass. Even this small fraction may be important, for organically bound tritium OBT can be incorporated completely as a precursor of biomass production (only non-exchangeable OBT). In practice a small fraction of OBT must be separated from a large amount of soil water. For that purpose the vacuum distillation is generally applied in our laboratory. After the first distillation step non-radioactive water is added again, before the distillation is repeated. It has been tested by employing a double labelling of the soil water (HTO and $H_2{}^{18}O$) that at least three or better more subsequent distillation steps are necessary to obtain a complete yield.

2. Results

The results of the German air samples exposed to the plume in France cannot be compared directly to the measurements of the same samples in the laboratory. The position of the sample containers exposed at point A(1) was not measured as exactly as necessary. But the deposition velocities measured in the laboratory and that calculated from the HTO content of the samples after exposure are comparable within each sample series.

The results of the soil cores collected at the French site demonstrate: The deposition velocity in forest soil is high and remains nearly constant (about 10^{-3} m s^{-1}). Those in field and meadow soil nearby are smaller by about one order of magnitude. Even large variations of the soil water content during the observation period did not diminish the deposition velocity drastically. The results confirm that the soil was in a bioactive state during the release day. The large differences between soils of various utilisations can be observed on the release day and immediately thereafter, too (field 0.6-3.6; meadow 0.9-2.5; forest 5.8-13.2 * 10^{-4} m s^{-1}). At point A(1) and some points of line B our data from laboratory measurements and calculations from field observations are in good agreement (deposition velocities calculated from integrated HT concentration in air and amount of HTO in the soil, observations by the other participants). For a soil sample collected at point A(1) a reemission rate of 2 % h^{-1} was measured (range of all soil samples: 1.4 - 7.8 % h^{-1}) . The reaction product HTO will be found in the upper soil layers (50 % 0.7 - 3.1 cm below soil surface). These results are in good agreement to our observations of three sites near KFA Jülich. There the forest soil is also the most active one during the whole year. Generally the deposition velocity of HT into a soil is mainly governed by the kind of its utilisation (field, forest or pasture) and its water content (except forest soil).

In the Canadian release experiment the variation of the deposition velocity across the area investigated was smaller than expected. This may be due to the relatively coarse sandy soil. The mean deposition velocity in the whole clearing and the adjacent forest was 6.6 * 10-4 m s^{-1} (laboratory method), the variation at the B-line in the range of about ± 10 %. No significant influence of the plant species or the plant cover at all could be detected (including nitrogen fixing plants). Reemission rates of 1.3 - 2.4 % h^{-1} were measured. Directly before the release the soil surface of an experimental plot was labelled with $H_2{}^{18}O$ in order to trace the movement of water. In the vegetation a first peak of the ^{18}O label occurs within some hours after the release, the next one during the subsequent morning. The leaf water has a remarkably high ^{18}O content. This indicates an intensive direct exchange of water vapour between soil and plant biomass, via the air humidity directly, and not via the internal transport system of the plant. The reemission was studied by exposing a set of 60 samples (200 g each). The HTO content decreases to about one third of its initial value from the afternoon of the release day during the next two days.

Thus HTO is continuously reemitted back into the adjacent air layer due to an isotopic exchange between soil water and the water vapour in the air.
In the laboratory test soil samples were first exposed to HT in order to label their surface with HTO and exposed to tritium-free air thereafter. The reemission rate increases continuously with an enhancement of the air turnover (up to about 4 m s^{-1}). Surprisingly the reemission rate decreases drastically during the observation period of 6 h, an effect which cannot be explained simply by the loss of HTO reemitted previously. The relative humidity of the air had no significant influence on the reemission rate. This may lead to the conclusion that the exchange process is limited mainly by the transport across the soil/air boundary. This assumption is supported by the observation that differences in the initial HTO profile do not influence the reemission.
The HTO/soil incubation experiments necessitate aerobic conditions, i.e. the samples must be supplied with fresh air. This results in a continuous loss of HTO by an exchange between soil water and air water vapour. Taking into account this loss, an uptake of tritium into the biomass of about 1 ‰ each week was observed (related to the HTO content of soil water). The biological synthesis and consequently the uptake of tritium can be stimulated by the addition of energy sources for soil biomass such as glucose. The enhanced uptake of tritium into OBT then corresponds well with the increased biological activity, measured as increase of soil respiration, i.e. carbon dioxide release from soil using an infrared analyser. The results indicate that no special pathway must be taken into account. Tritium as HTO from the soil water pool will be incorporated via the common pathways of biosynthesis.

3. Conclusions:

The release experiments have demonstrated that our laboratory method (non-disturbed soil cores exposed to HT in a gas circuit) is able to predict the deposition velocities under natural conditions. A large area including soils of different use and utilisation can be characterized quickly. Forest soil is very active in fixing HT independently on its water content. The results of the French release experiment represent a rural area, whereas the Canadian site was a special case (coarse sandy soil, arteficially changed). The reemission of HTO as an important source of the more radiotoxic HTO is about 1 - 8 percent per hour, decreasing with time after exposure. The decrease is more rapid than one would expect by simple loss of HTO during the procedure (perhaps due to microdiffusion from the reaction sites).
The rapid exchange between soil water and air water vapour may also lead to a larger HTO concentration in leaf water as expected from root uptake and transport in the xylem only.
The uptake pathway of tritium from the reaction product HTO into the biomass of the soil so far no specific biosynthetic pathway could be elucidated until now. Only after stimulating iosynthesis general by the addition of glucose, the OBT and the CO_2 release from the soil increase in parallel.

IV. Objectives for the next reporting period:

Study of the reemission under natural conditions including the diurnal rhythm and different weather situations using ^{18}O-labelled water too. Depth profiles of biological HT/HTO-conversion activity. Isotopic effect between H,D and T to study the mechanism of deposition (limitation by diffusion or reaction). Linkage between HT uptake and nitrogen fixation. Influence of time and conditions of storage of soil cores. Deposition velocities of typical German representative soils from different locations focussing on their status (water content, organic material). Participation in the second French release experiment ? First steps of modelling the HT/HTO pathways in the natural ecosystem.

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

Dr. C.D. Burnham, Canad. Fusion Fuels Technology Proj., 2700 Lakeshore Road West, Mississauga, Ontario L5J 1K3, Canada
Dr. B. Brown, Atomic Energy of Canada Ltd., Chalk River Nuclear Laboratories Chalk River, Ontario, KOJ 1J0, Canada
Dr. H. Djerassi, CEA / CEN Saclay, DPT / SPIN, bâtiment 393, F - 91 191 Gif sur Yvette Cedex, France
Dr. Belot, CEA/CEN Fontenay aux Roses, IPSN/DERS/SERE, F 92 260 Fontenay aux Roses, France
Dr. Fred S. Spencer, Dr. G. Ogram, Ontario Hydro, 800 Kipling Ave., Toronto, Ontario, M8Z 5S4, Canada
Dr. Gulden, The NET TEAM, MPI für Plasmaphysik, 8046 Garching
Frau Dr. Diabate, KfK Karlsruhe, Zentralabt. Sicherheit, Radioökologie, Postfach 3640, 7500 Karlsruhe
Dr. Bunnenberg, Niedersächs. Inst.für Radioökologie an der Universität Hannover, Herrenhäuser Strasse 2, 3000 Hannover

VI. Publications:

1. Publications:

Förstel, H., Messung der zeitlichen und räumlichen Variation der HT-Depositionsgeschwindigkeit in der Umgebung der KFA Jülich. Fachgespräch Überwachung der Umweltradioaktivität, Bundesminister Umwelt, Naturschutz und Reaktorsicherheit, Bonn 1987, p. 475 - 496.

Förstel, H., Deposition velocity of elementary tritium in soil of different use. CEC Seminar The Cycling of Long-lived Radionuclides in the Biosphere: Observations and Models. Vol. 2, Madrid 1986, p. 1 - 6.

Förstel, H., Confirmation of laboratory results by HT releases under field conditions: HT deposition velocity and reemission rate. CEC Workshop Methods of Assessing Reliability of Environm. Transfer Model Predictions, Athens 1987, in press.

Förstel, H. , Trierweiler, H., Results of the contribution of the Nuclear Research Centre Jülich (KFA) to the French Release Experiment FREX I.- Final Report, CEA Paris, in press.

2. Internal Reports:

Förstel, H. , Trierweiler, H., Contribution of the Nuclear Research Centre Jülich to the French Release Experiment FREX I.-Preliminary Rep., CEA SE1.87.02, Paris 1987. .

Lepa, K., Modellversuche zur HTO-Reemission gewachsener Böden nach HT-Exposition. Diplom FH Aachen/Abt. Jülich, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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. Galvão
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
- L. Canelas
- M.J. Madruga
- A. Brogueira
- M.M. Sequeira
- M.M. Brito
- J.A. Corisco

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:

The objectives for the year 1987 were the physico-chemical characterization of the water, sediments and biological material, the mineralogical study of the of the phytocenoses, a limnological study and the prosecution of the radioactivity measures. It was also intended to finish the experiments with Cs-134 in sediments and in the Trophic chain.

In what concerns the modelling, the objective was the installation of the mathematical model.

III. Progress achieved:

1. Methodology

Radioecology

The trophic chain considered was: the micro-Algae Selenastrum capricornutum Prinz, the Crustacean Daphnia magna Straus and the Fish Tinca tinca Linnaeus, using water from Fratel dam. Simultaneously water-sediment interactions were studied using as well water and sediments from Fratel dam.
The radionuclide used was Cs-134 and the detection system consists of a well type NaI(Tl) detector connected to a single channel analyser.

Modelling

A comprehensive bibliography was consulted, and a comparison of the performences of different models was done. The aim was to find a model whose characteristics should indicate it as the appropriate one for simulation of hydrologic and hydraulic parameters of the river.
Field work for sampling and measuring local parameters over the river Tejo.

2. Results

Radioecology

2.1 Uptake and retention of Cs-134 in Selenastrum capricornutum Printz.

The experiments with this unicelular Algae have been repeated and therefore the previous results have been improved. The higher concentration factor (CF), was obtained in non-enriched Fratel water, 1347±133, referred to dry weight.
The loss experiments in confined medium (Fratel water) are expressed by the following retention function: $R_t=0.65e^{-76.37t} + 0.35e^{-1.76t}$, with two biological half-lives: $Tb_1=0.22$ hours and $Tb_2=9.36$ hours.

2.2 Uptake and retention of Cs-134 in Daphnia magna Straus.

The uptake experiments directly from labelled river water have been carried out either with the small Crustaceans, in starvation, (CF=53±4) or being intermittently immersed into an inactive S. capricornutum culture, (CF=29±3).

In the uptake experiments through contaminated food, the transfer factor (TF), is defined as the ratio specific activity of D. magna /specific activity of S. capricornutum. The TF at the end of 23 days had the value of 4.05, being represented by the logarithmic function, TF=0.65+1.09 ln t.

In the simultaneous accumulation through water and food it was observed (paralel experiments) that the global contamination of D. magna is higher than the contamination by water. The TF estimated is also represented by a logarithmic function: TF=-6.86+4.20 ln t.

When the D. magna has been contaminated through the water, the retention showed two biological half-lives, Tb_1=0.12 days and Tb_2=1.80 days, being expressed by the following expression: $R_t=0.281e^{-5.640t} + 0.719e^{-0.385t}$.

While the contamination was through the food (labelled S. capricornutum), the retention was expressed by: $R_t=e^{-0.410t}$, with only one biological half-live, Tb=1.7 days.

Both loss experiments lasted 14 days.

2.3 Uptake and retention of Cs-134 by Tinca tinca Linnaeus.

In the experiments of direct accumulation from water, Tinca tinca was kept in starvation, and after 32 days the estimated CF followed the function $CF=0.39t^{0.70}$.

The accumulation through the food chain is still running.

The retention was only studied in the first case and two biological half-lives were found, Tb_1=8.7 days and Tb_2=86.6 days. The retention function is $R_t=0.22e^{-0.08t}+0.75e^{-0.008t}$

2.4 Sorption and desorption of Cs-134 in sediments.

Different sediment concentrations, ranging from 0.5 to 5 $mg.ml^{-1}$, (< 500 μm), were continously stirred, during about 20 days. For an initial Cs-134 contamination lower than 10^4Bq and for sediment concentrations from 0.5 to 5 $mg.ml^{-1}$, the K_d ranged from $4.0x10^3$ to $5.7x10^3$ $ml.g^{-1}$. While, for an initial contamination higher than 10^4Bq, and for the same sediment concentrations the K_d ranged from $1.8x10^3$ to $3.4x10^3$ $ml.g^{-1}$. In the first case, the K_ds increase slowly with the sediment concentration: y=4176+307 x. Otherwise, for the initial Cs-134 contamination 10^4Bq, the K_ds increase with the sediment

concentration, but for sediment concentrations higher than 2 $mg.ml^{-1}$ there is a trend to equilibrium.

The kinetics of the distribution coefficient evolution with time, is a logarithmic function.E.g., for the concentration of 1 $mg.ml^{-1}$ the expression is K_d=1091+271 ln t.

The compartmental analysis of uptake curve, was made through the curve representing Cs-134 activity in solution in % with time.For the 1 $mg.ml^{-1}$ sediment concentration the following exponential was estimated:$y=34.0\ e^{-22.339} + 23.6\ e^{-2.961t} + 41.9\ e^{-0.087t}$. The half-time periods determined were respectively 44.7 min., 5.6 hours and 8.4 days.

The same compartmental analysis in desorption experiments, gave the half-time periods of 26.7 min, 18.4 hours and 7.2 days, in an open system, and 3.9 min. and 2.2 days, in a closed system.

In the desorption experiments, transfer factors water-sediment were determined at different sediment concentrations. In a closed system, all the results are similar and the mean value for the Cs-134 activity retention (%) in sediments is 89.5±5.8.

Modelling

A lot of parameters of river Tejo were collected and analysed for an increased knowledge about its hydrologic and hydraulic characteristics.

A model which can operate as a steady state or as a dinamic one, allowing for multiple waste discharges, whitedrawals and tributary flows, for the simulation of several water quality constituents was installed, and many runs and tests are being carried out.

3. Discussion

Radioecology

In what concerns this trophic chain, it is still necessary to carry out the experiment of the simultaneous contamination of fish through water and food, to have a global idea of the contamination processes in fish.

However concerning the primary consummer it is possible to say that the water is the main contamination pathway.

The primary producer reaches the equilibrium within the first 24 hours, but it also looses very quickly the activity (83% within 24 hours) when reared in inactive water.

Concerning the water-sediments interaction, the compartmental analysis made possible to interprete the Cs-134 uptake and release mechanisms in sediments. From the desorption experiments with different sediment concentrations, the estimated Cs-134 transfer factors range from 0.95×10^{-4} to 1.9×10^{-4} and they are comparable to those calculated in situ, Fratel dam, $2.4\ 10^{-4}$ $g.ml^{-1}$.
We think that the planned objectives for the period were completely accomplished.

Modelling

In general way it is possible to say that the objectives for the period were accomplished.

IV. Objectives for the next reporting period:

Two more seasonal samplings (Winter and Summer) to accomplish the whole characterization of the river. Experiments concerning the Co-60 transfer trough a simplified Trophic chain from the Fratel dam and the interactions water-sediments from the Fratel dam too.
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.

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:

-Étude expérimentale du comportement du ^{134}Cs en sediments d'eau douce.
M. José Madruga et M. Carolina Vaz Carreiro
à presenter au IVème Symposium International de Radioécologie "Impacte des Accidents d'Origine Nucléaire sur l'Environment", Cadarache, France, 14-19 Mars 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.
à presenter á la 1ª Conferência Nacional sobre a "Qualidade do Ambiente", Aveiro (Portugal), 22-24 Février 1988.

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 coastel areas and which are significant in the population diet.

II. Objectives for the reporting period:

Determination of ^{210}Pb, ^{210}Po, $^{239+240}Pu$ and ^{241}Am in sea-food samples collected at the Portuguese offshore and at Madeira island, including deep coastel waters.

III. Progress achieved:

1. Methodology

Analysis of ^{210}Po are performed through the classical procedure of spontaneous plating of ^{210}Po together with ^{209}Po isotopic tracer onto a silver disk from hydrocloric solution. A second ^{210}Pb plating a few month 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 spectromerty.

2. Results

The α-emitter ^{210}Po (T1/2=138.4d) is a widespread natural radionuclide coming from the decay of ^{210}Pb through the ^{210}Bi. Its concentration in coastalmarine waters roughly varies between 1-4 $mBq.L^{-1}$ Marine biota is able to concentrate ^{210}Po to high levels, as 10^3-10^5 relative to sea water concentration, whilst ^{210}Pb is discriminated, thus allowing higher $^{210}Po/^{210}Pb$ ratios than in sea water. Po-210 concentrations in mixed zooplankton reaches 34-51 $Bq.kg^{-1}$ (fresh wt), special groups as copepods reaching even higher concentrations ~90 $Bq.kg^{-1}$, whereas gelatinous zooplankton display ~1 $Bq.kg^{-1}$. Epipelagic teleosts feeding on plankton dislayed the highest concentrations found in fish muscle, 2-21 $Bq.kg^{-1}$. Contrasting with this, demersal teleosts and elasmobranchs display lower ^{210}Po concentrations, ranking from o.5-7 $Bq.kg^{-1}$ respectively. Much higher concentrations can however be measured in fish liver, gonad, bone and piloric caecca, and small mesopelagic fish can reach ~800 $Bq.kg^{-1}$ on a whole-body basis. Due to these ^{210}Po activity concentrations, dose equivalent rates delivered to biological tissues in marine organisms can vary from 0.4 to 50 $mSv.y^{-1}$ in zooplankton, and from 0.1 $mSv.y^{-1}$ in the muscle of bathypelagic fish to 6 $mSv.y^{-1}$ in the muscle of epipelagic teleosts, and xe

so high as 300 $mSv.y^{-1}$ (30 $rem.y^{-1}$) in the liver and 5.6×10^3 $mSv.y^{-1}$ (5.6×10^2 $rem.y^{-1}$) in the gut wall of sardines. It is concluded that even in a stable physical environment, as the deep layers of the ocean, radiation dose regime is not uniform and it can be orders of magnitude higher than expected from the external radiation alone. Moreover, in organisms living in the same ocean layer a wide range of internal radiation doses exists and it is essentially sustained by ^{210}Po food-chain transfer.

Some fishes collected at Madeira were equally analysed for $^{239+240}Pu$ and ^{241}Am. The analyses were mainly performed on muscle samples, but some other organs were also analysed. The results obtained up to now confirm that deep-sea pelagic fish show, in general, lower concentrations for these radionuclides than fish living in shallower waters. The concentrations in muscle of deep-sea pelagic fish (Aphanopus carbo, - 1200m and Alepocephalus bairdii, - 1600 m) range from 0.03 to 0.9 $mBq.kg^{-1}$ (with an isolated value of 1.9) for $^{239+240}Pu$ and from 0.22 to 0.39 $mBq.kg^{-1}$ for ^{241}Am. In different species of fish living at depths down to 600 m, $^{239+240}Pu$ concentrations range from 0.8 to 5 $mBq.kg^{-1}$ and ^{241}Am from 0.2 to 6 $mBq.kg^{-1}$.

3. Discussion

Results on ^{210}Po concentration in marine fauna confirm its role as the main internal α-emitter. Radiation doses from ^{210}Po alone are much higher than radiation doses coming from other natural and man-made α-emitters. The transfer of unsupported ^{210}Po along marine food-chains is now well documented and highlights that very different radiation dose regimes are experienced by organisms living in the same physical environment.

The progress achieved may, in general, be considered on line with the objectives for the reporting period, although the number of transuranics analyses has been quite reduced due to severe damage in the multichannel analyser.

IV. Objectives for the next reporting period:

Enlargement of ^{210}Po - ^{210}Pb analysis to deep sea fauna and their transfer along marine food-chains.
Comparison of ^{210}Po - ^{210}Pb cycling in marine and terrestrial food-chains.
Analysis of U, Th and Ra isotopes in marine biota.
Continuation of analyses of ^{239}Pu and ^{241}Am in marine biota and starting of measurements in seawater.

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, Lisbon 7-12 Dec. 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-037-F

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

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

Dr A. Grauby
SERE-DERS
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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 seront menées selon deux approches : l'une in situ, l'autre expérimentale.

II. Objectives for the reporting period:

Nous avons étudié le rôle de certains paramètres pouvant entraîner la formation dans l'eau de mer de formes physicochimiques particulaires autres que la forme soluble cationique NpO_2^+. Il s'agit de la concentration en neptunium, de la salinité et du pH.
Afin d'améliorer la mesure du 237-Np dans l'eau de mer, nous avons effectué une étude expérimentale de l'adsorption du Np en fonction du pH sur des supports carbonatés, des hydroxydes de fer, du bioxyde de manganèse.
En utilisant l'analyse par activation neutronique (à laquelle nous avons apporté quelques modifications), nous avons dosé 237-Np dans 18 espèces littorales afin de définir des bioindicateurs et les potentialités de fixation le long des chaînes trophiques.

III Progress achieved

Un précedent travail réalisé par GERMAIN et al. (1987) a montré, en utilisant le 239-Np comme traceur, que le neptunium à une concentration molaire de 10^{-12}M au moment de l'introduction du marqueur dans l'eau de mer, est essentiellement sous forme cationique peu chargée (NpO_2^+) (83 %) il présente également une faible proportion de formes anioniques (5 à 8 %) et de formes particulaires (9 %).

Au cours de l'année 1987 l'accent a été mis sur la distribution des formes particulaires du Np en fonction de 3 paramètres : la salinité du milieu, le pH, la concentration en Neptunium.

Les résultats sont exprimés en % de Np particulaire retenu sur des membranes filtrantes de 0,22 µm de diamètre de pores par rapport au Np total introduit dans la solution :

- en fonction de la salinité, le neptunium étant à la concentration 10^{-12} $M1^{-1}$ après 5 jours de contact du 239-Np avec le milieu aqueux.

S ‰	S=30	S=21	S=18	S=15	S=12	S=9	S=6	S=3	S=0
% de 239-Np particulaire	8	7,2	7,2	6,6	3,6	3,6	2,9	3,8	1,3

- en fonction du pH, dans une eau de mer de salinité 30 ‰ et une concentration en neptunium de 5.10^{-12} $M1^{-1}$ après 4 heures de contact du Np avec la solution

pH	2,0	3,2	4,3	4,8	5,8	6,3	7,5	8,3	9,1
% de 239-Np particulaire	8,6	8,3	6,9	4,7	8	13,3	10,9	17,7	17,5

- en fonction de la concentration en Np

Le marqueur étant le 239-Np la variation en concentration du Neptunium est obtenue par ajouts de 237-Np dans des domaines de concentration allant de 10^{-12}M l^{-1} à 10^{-7} M l^{-1}. Les résultats sont exprimés après 15 heures, 5 jours et 10 jours, de contact du neptunium avec l'eau de mer à la salinité 30 ‰

de 239-Np particulaire	$10^{-12}M1^{-1}$	$10^{-11}M1^{-1}$	$10^{-10}M1^{-1}$	$10^{-9}M1^{-1}$	$10^{-8}M1^{-1}$	$10^{-7}M1^{-1}$
t = 15 heures	2,8	19,3	24,6	22,1	23,9	17,6
t - 5 jours	8	15	22,9	28,3	27,6	26,3
t - 10 jours	13	31,5	33,6	28,5	30,9	30,7

Une seconde partie du travail effectué en 1987 a porté sur l'adsorption du 239-Np, à la concentration de 10^{-12} $M1^{-1}$, sur l'hydroxyde ferrique, le bioxyde de manganèse et le carbonate de calcium, en fonction du pH d'une eau de mer de salinité 30 ‰. Les résultats sont présentés sur la figure 1 et exprimés en pourcentage de 239-Np fixé sur l'adsorbant.

Les 3 courbes de la figure 1 présentent un point d'inflexion bien marqué entre pH 7 et pH 8 independant du support adsorbant. Il semble que cette évolution corresponde à un phénomène lié à la physicochimie propre du neptunium dans cet intervalle de pH en milieu eau de mer.

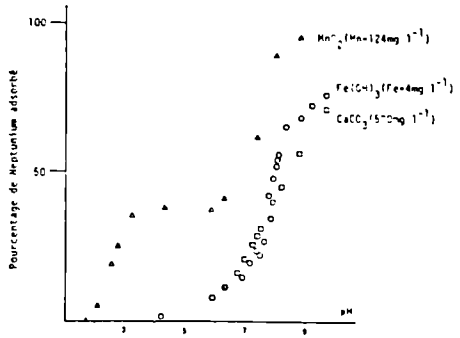


Figure 1 - Rendements d'adsorption du ^{239}Np sur MnO_2, $Fe(OH)_3$ et $CaCO_3$ en fonction du pH dans un milieu eau de mer de salinité 30 ‰

De janvier à mars 1986 des échantillons représentatifs des espèces vivant sur les côtes du Nord-Cotentin ont été prélevés à Goury, afin de doser le 237-Np, de dégager les espèces indicatrices et d'établir une hiérarchie trophique.
L'analyse par activation neutronique a été utilisée pour doser le 237-Np (analyses assurées par le Laboratoire Pierre Süe de Saclay). Cette technique a été publiée en 1987 (cf. GERMAIN et al.) Quelques modifications ont été récemment apportées à la technique. En particulier, dans le cas de l'eau de mer une préconcentration a été réalisée, le neptunium étant précipité par du bioxyde de manganèse, obtenu à partir de 150 mg de permanganate de potassium, à pH 9,sur des échantillons de 20 litres d'eau de mer. Les rendements sont proches de 90 %.
Nous avons calculé le rapport R=activité de l'organisme frais en mBq kg^{-1}/ activité de l'eau de mer en mBq kg^{-1} (Tableau I). Du fait de la difficulté de l'analyse et de son coût, seulement 2 échantillons d'eau de mer ont été traités, les résultats étant 0,028+0,008 mBqkg^{-1} et 0,011 + 0,034 mBqkg^{-1}. Les valeurs de l'eau de mer n'ont qu'une valeur ponctuelle dans le temps mais elles permettent de calculer le rapport R et de dégager les potentialités respectives des espèces à fixer le 237-Np.

Lichen	60 à 150	Ascidies	400 à 1100
Algues rouges	40 à 6000	Annélides	200 à 500
Algues brunes	20 à 300	Mollusques herbivores	100 à 600
Algues vertes	250 à 650	Mollusques carnivores	40 à 250
Phanérogames	100 à 300	Crustacés carnivores	10 à 400
Eponges	500 à 1200	Poissons	ND à 100

Il est difficile de définir une hiérarchie relative à la fixation du 237-Np en fonction des niveaux d'organisation. Parmi les espèces étudiées, certaines algues rouges fixent bien 237-Np, en particulier Corallina officinalis qui possède une structure calcifiée. Ces algues sont suivies par une éponge, une ascidie Dendrodoa grossularia. Cette dernière espèce, bien qu'appartenant à un groupe zoologique évolué, possède un mode de nutrition par filtration de particules qui l'apparente aux invertébrés inférieurs, et ce mode de nutrition explique peut être la teneur en 237-Np. Ensuite dans l'ordre décroissant des possibilités de fixation, nous trouvons les mollusques herbivores, les crustacés carnivores, puis les poissons.
De cette étude nous pouvons dégager quelques espèces indicatrices ; c'est à dire des espèces assez facilement obtenues et qui fixent correctement 237-Np : les algues rouges Corallina officinalis, Chondrus crispus (algue ayant un intérêt industriel), ainsi que les mollusques bivalves Patella sp. et Gibbula umbilicalis. Il est à noter que les algues brunes, telles les fucus, souvent utilisées comme espèces indicatrices des radionucléides artificiels fixent peu le 237-Np.

Echantillon	Poids sec / Poids frais	mg kg^{-1} frais	mBq kg^{-1} frais	R = rapport activité de l'organisme frais en mBq kg^{-1}/activité de l'eau de mer en mBq kg^{-1}	
				eau de mer février 1986	eau de mer mai 1986
- Lichen					
Lichina pygmea 27/1/1986	0,47	$6,6 \pm 1,4 \times 10^{-8}$	$1,7 \pm 0,4$	61 ± 22	155 ± 60
- Chlorophytes					
Enteromorpha sp. 29/1/1986	0,25	$27,5 \pm 5 \times 10^{-8}$	$7,2 \pm 1,3$	257 ± 87	655 ± 234
- Phaeophytes					
Fucus serratus 27/1/1986	0,14	$3,6 \pm 0,7 \times 10^{-8}$	$1,0 \pm 0,2$	36 ± 13	91 ± 34
Laminaria digitata 27/1/1986	0,12	$2,2 \pm 0,6 \times 10^{-8}$	$0,6 \pm 0,2$	21 ± 9	55 ± 25
Cystoseira tamariscifolia 27/1/1986	0,14	$12,6 \pm 2,5 \times 10^{-8}$	$3,3 \pm 0,7$	118 ± 42	300 ± 112
- Rhodophytes					
Corallina officinalis 27/1/1986	0,38	$247,0 \pm 49,0 \times 10^{-8}$	$64,3 \pm 12,9$	2296 ± 802	5845 ± 2154
Chondrus crispus 27/1/1986	0,17	$68,0 \pm 13,6 \times 10^{-8}$	$17,7 \pm 3,5$	632 ± 220	1609 ± 590
Porphyra umbilicalis 29/1/1986	0,12	$4,8 \pm 1,0 \times 10^{-8}$	$1,3 \pm 0,3$	46 ± 17	118 ± 46
- Phanerogames					
Zostera marina 11/2/86	0,10	$13 \pm 3,0 \times 10^{-8}$	$3,4 \pm 0,8$	121 ± 45	309 ± 20
- Spongiaires					
Halichondria panicea 27/1/1986	0,13	$53,3 \pm 10,4 \times 10^{-8}$	$13,9 \pm 2,7$	496 ± 171	1264 ± 461
- Tunicier					
Dendrodoa grossularia 27/1/1986	0,17	$47,6 \pm 10,2 \times 10^{-8}$	$12,4 \pm 2,7$	443 ± 159	1127 ± 426
- Annélides polychaetes					
Arenicola marina 5/2/1986	0,15	$22,5 \pm 4,5 \times 10^{-8}$	$5,9 \pm 1,2$	211 ± 74	536 ± 198
- Mollusques gastéropodes					
Patella vulgata chair	0,15	$15,0 \pm 3,0 \times 10^{-8}$	$3,9 \pm 0,8$	139 ± 49	355 ± 132
" " coquille	0,94	$10,3 \mp 1,9 \times 10^{-8}$	$2,7 \mp 0,5$	96 ∓ 33	245 ∓ 88
28/1/1986					
Globula umbilicalis					
chair	0,25	$25,0 \pm 5,0 \times 10^{-8}$	$6,5 \pm 1,3$	232 ± 81	591 ± 216
coquille	0,86	$25,8 \mp 5,2 \times 10^{-8}$	$6,7 \mp 1,6$	239 ∓ 89	609 ∓ 238
28/1/1986					
Nucella lapillus chair	0,24	$4,8 \pm 1,0 \times 10^{-8}$	$1,3 \pm 0,3$	46 ± 17	118 ± 46
coquille	0,96	$10,8 \mp 2,0 \times 10^{-8}$	$2,8 \mp 0,5$	100 ∓ 34	255 ∓ 91
28/1/1986					
- Crustacés décapodes					
Carcinus maenas carapace	0,83	$18,3 \pm 3,3 \times 10^{-8}$	$4,8 \pm 0,9$	171 ± 58	435 ± 159
hépatopancréas	0,21	$6,3 \mp 1,3 \times 10^{-8}$	$1,6 \mp 0,3$	57 ∓ 19	145 ∓ 52
chair	0,16	$0,5 \mp 0,2 \times 10^{-8}$	$0,2 \mp 0,1$	7 ∓ 4	18 ∓ 11
branchies	0,08	$2,4 \mp 0,7 \times 10^{-8}$	$0,6 \mp 0,2$	21 ∓ 9	55 ∓ 25
30/1/1986					
Cancer pagurus					
carapace	0,72	$4,4 \pm 1,3 \times 10^{-8}$	$1,1 \pm 0,3$	39 ± 15	100 ± 41
hépatopancréas	0,22	$3,1 \mp 0,7 \times 10^{-8}$	$0,8 \mp 0,2$	29 ∓ 11	73 ∓ 29
chair	0,16	$0,7 \mp 0,2 \times 10^{-8}$	$0,2 \mp 0,1$	7 ∓ 4	18 ∓ 11
branchies	0,10	$2,4 \mp 0,5 \times 10^{-8}$	$0,6 \mp 0,1$	21 ∓ 7	55 ∓ 19
12/3/1986					
- Poisson littoral					
Blennius pholis chair	0,19	$< 1,0 \times 10^{-8}$	$< 0,3$	-	-
peau	0,12	$< 1,2 \times 10^{-8}$	$< 0,3$	-	-
restes	0,23	$< 1,6 \times 10^{-8}$	$< 0,4$	-	-
branchies	0,17	$< 5,1 \times 10^{-8}$	$< 1,3$	-	-
foie	0,06	$< 0,6 \times 10^{-8}$	$< 0,2$	-	-
nageoires	0,15	$< 1,5 \times 10^{-8}$	$< 0,4$	-	-
viscères	0,09	$4,8 \pm 0,9 \times 10^{-8}$	$1,2 \pm 0,2$	43 ± 7	109 ± 39
arêtes	0,23	$< 6,0 \times 10^{-8}$	$< 1,6$	-	-
30/1/1986					

TABLEAU I : Niveaux du ^{237}Np dans des échantillons marins prélevés à Goury, près de la Hague (erreur relative 20 %). et valeurs du rapport : activité de l'organisme frais en mBq kg^{-1}/activité de l'eau de mer en mBq kg^{-1}.
1 mBq = 0,027 pCi

IV. Objectives for the next reporting period:

Nous aborderons l'étude de la complexation du Np(V), avec EDTA, les acides fulvique, glutonique, aspartique, malique, alginique, et la décomplexation du neptunium par les ions cuivre. Les transferts de neptunium à partir de l'eau de mer vers des espèces de mollusques lamellibranches et gastéropodes filtreurs, carnivores et brouteurs seront étudiés. In situ, à l'aide d'un bioindicateur, nous étudierons la dispersion de 237-Np autour du Cotentin.

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:

1. GERMAIN, P., GANDON, R., MASSON, M. & GUEGUENIAT, P. 1987. Experimental studies of the transfert of neptunium from Sea Water to sediments and organisms (Annelids and molluscs). J. Environ. Radioactivity, 5, 37-55.
2. GERMAIN, P., GUEGUENIAT, P., MAY, S. & PINTE, G. 1987. Measurement of transuranic elements, chiefly 237-Np (by neutron activation analysis), in the physical and biological compartments of the french shore of the english Channel. J. Environ. Radioactivity, 5, 319-331.

Title of the 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 caractétistiques 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 sur 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 premières expériences ont eu pour objectif d'étudier l'influence de la taille des particules sur l'efficacité de leur mise en suspension dans l'atmosphère.

III. Progress achieved:

Aux cours des années précédentes nous avions montré expérimentalement que les aérosols obtenus par éclatemement de bulles à la surface de l'eau de mer étaient beaucoup plus riches en métaux lourds, et particulièrement en américium, que l'eau de mer dont ils étaient issus. En opérant avec de l'eau de mer filtrée de manière conventionnelle, nous avions obtenu des facteurs d'enrichissement très faibles. Ces facteurs augmentaient de façon importante lorsqu'on introduisait des particules minérales fines (oxydes d'aluminium ou de fer) dans l'eau de mer préalablement filtrée. Le facteur d'enrichissement obtenu était d'autant plus important que les particules étaient plus fines. Il était néanmoins difficile de quantifier l'influence de la taille des particules, car les particules introduites dans l'eau de mer coagulaient rapidement et étaient remplacées par des agrégats polydispersés de taille plus importante. En 1986, nous avons tenté de remplacer les ajouts de particules minérales par des ajouts de particules de latex (polystyrène) , mais il est apparu que ces particules coagulaient dans l'eau de mer tout aussi rapidement que les particules minérales précédemment utilisées et que nous devions, avant toute simulation, résoudre le problème de la stabilisation de particules fines dans un milieu à charge saline élevée tel que l'eau de mer.

Méthode expérimentale

La coagulation des particules dans une solution saline concentrée peut être ralentie, voire supprimée, si la surface des particules est enduite de polymères flexibles qui forment un manteau autour de chaque grain. Nous avons utilisé des particules monodispersées de polystyrène marquées par un traceur fluorescent et enduites d'albumine. Les particules ainsi traitées ne s'agglomèrent pas au contact de l'eau de mer et constituent un modèle approprié des particules naturellement présentes en eau de mer.

Le mode opératoire consiste à introduire 1 à 10 mg de particules monodispersées, de diamètre 0,1 - 0,6 - 1 ou 6.8 micron, dans 15 ml d'une solution contenant 1.5 mg d'albumine. Une partie de l'albumine s'adsorbe irréversiblement sur les particules, l'excédent d'albumine peut être éliminé par diafiltration. Les particules ainsi préparées sont introduites dans 1 litre d'eau de mer préalablement filtrée. Cette suspension est ensuite introduite dans le dispositif de bullage précédemment mis au point, et décrit dans une publication antérieure (Atmospheric Environment vol. 16, 1463-1466, 1982). Les aérosols produits par bullage sont recueillis sur un filtre. Les quantités de particules contenues dans l'échantillon d'eau de mer et celles mises en suspension et recueillies sur

filtre sont mesurées par fluorimétrie. Le sodium mis en suspension est déterminé par potentiométrie au moyen d'une électrode spécifique. Le facteur d'enrichissement des embruns en particules est égal au rapport des concentrations de ces particules dans l'eau des embruns et dans l'eau de mer dont ils sont issus. Le volume d'eau mis en suspension sous forme d'embruns est obtenu à partir de la quantité de sodium présente dans les aérosols et recueillie sur filtre.

Résultats

Les premiers essais ont été effectués avec 10 mg/l de particules monodispersées de 0,1 - 0,6 - 1 et 6,8 micron, enduites d'albumine suivant la méthode précédemment exposée. Des mesures, effectuées au moyen d'un granulomètre à laser, nous ont montré que les particules ainsi traitées restaient bien individualisées et ne s'agglomeraient pas au contact de l'eau de mer.

Les facteurs d'enrichissement obtenus sont donnés sur la courbe suivante, qui nous montre que ce facteur augmente de 160 à 910 pour des particules dont la taille diminue de 6.8 à 0,1 micron. La méthode de simulation utilisée ici semble tout à fait appropriée à l'étude de la mise en suspension des particules contenues dans l'eau de mer.

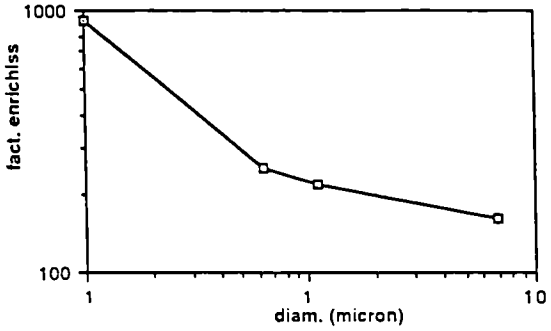


Fig 1 : Enrichissement des embruns en particules. Les embruns ont été obtenus par bullage d'air à travers un échantillon d'eau de mer contenant 10 mg/l de particules monodipersées.

IV. Objectives for the next reporting period:

La méthode de simulation mise au point en 1987 sera utilisée pour étudier plus complètement l'influence de la taille et de la concentration des particules en suspension dans l'eau de mer sur leur mise en suspension dans l'atmosphère. Une document rassemblant l'ensemble des résultats sera rédigé en 1988 et proposé pour publication.

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

VI. Publications:

Title of the project no : 3
Behaviour of radionuclides in marine, freshwater and terrestrial environments.
Radioecology of Continental Waters.
Thème général : 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., NUCHO, R., REMILLET, J.N. CALMET,D GONTIER. g, DIMIGLIO. 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 sédiment, de végétaux aquatiques et de poissons.

- Evaluer l'impact des centrales nucléaires de différentes filières et d'une usine de retraitement de combustible. A cela s'ajoute, maintenant l'impact des retombées consécutives à l'accident de Tchernobyl.

- Mettre au point des modèles expérimentaux pour étudier les mécanismes de transfert du ^{60}Co.

II. Objectives for the reporting period:

- Nous avons poursuivi nos campagnes de prélèvements et de mesures sur l'ensemble du Bassin Rhôdanien. Un bilan quantitatif et qualitatif permet d'évaluer l'impact des différents termes sources et en particulier celui de Tchernobyl.

- L'étude du transfert du ^{60}Co de l'eau vers le sédiment et du sédiment vers les larves de chironomes est terminée.

- Les travaux relatifs au transfert du ^{60}Co vers une algue planctonique (Scenedesmus obliquus) ont été poursuivis avec notamment l'étude de l'influence du stade de développement de la culture.

- Une étude de la bioaccumulation par la carpe du ^{60}Co contenu dans différents types de nourriture a été engagée.

III. Progress achieved:

Etude du Bassin Rhodanien

- Etude de terrain

Pour les 4 compartiments de l'écosystème fluvial (eau, sédiments, végétaux, poissons) la radioactivité artificielle reflète les différents termes sources.

Radioactivité artificielle (hors tritium) en fonction des termes sources dans les différentes zones	Eau $Bq.l^{-1}$	Sédiment $Bq.kg^{-1}$ sec	Végétaux $Bq.kg^{-1}$ sec	Poissons $Bq.kg^{-1}$ frais
Retombées	0,003	9	10	1,5
Centrales	0,02	40	70	4
Marcoule	0,5	1000	2000	20
Tchernobyl (Mai 1986)	0,5	600 à 1500	1700 à 4000	10 à 40

Après Tchernobyl les radionucléides à vie courte ont disparu en quelques semaines. Les césiums et les ruthéniums sont présents sur l'ensemble du fleuve à des niveaux parfois supérieurs à ceux qui existaient auparavant en aval de Marcoule, masquant l'effet de plusieurs années de rejets liquides des installations nucléaires.

Les prélèvements en 1987 montrent la disparition du ^{103}Ru, une décroissance rapide du ^{106}Ru et beaucoup plus lente pour les césiums 134 et 137.

Ce bilan et ces comparaisons n'ont été possibles que grâce à un suivi radioécologique régulier depuis plus de treize ans sur l'ensemble du fleuve. Les résultats de milliers de mesures sont gérés par une base de données relationnelle qui permet d'évaluer rapidement, de façon fine et fiable toute modification de la radioactivité des compartiments du Rhône, et dans tous les secteurs du fleuve.

- Transfert du ^{60}Co de l'eau vers le sédiment et du sédiment vers des larves de chironomes.

Le cobalt introduit dans la phase aqueuse est rapidement piégé par la masse sédimentaire. On observe une désorption significative quand le sédiment est mis en contact avec de l'eau inactive.

Le transfert du ^{60}Co du sédiment vers les larves de chironomes atteint un équilibre en 7 jours avec un facteur de transfert de 0,1. En l'absence de matière organique, la fixation est plus lente et moins importante. Le processus de décontamination met en évidence deux compartiments, le premier ayant une période de 1 jour ("vidange" du tube digestif), le deuxième correspond à une période longue de plusieurs jours.

La désorption du ^{60}Co par des larves contaminées à partir de sédiment dépourvu de matière organique est importante et rapide.

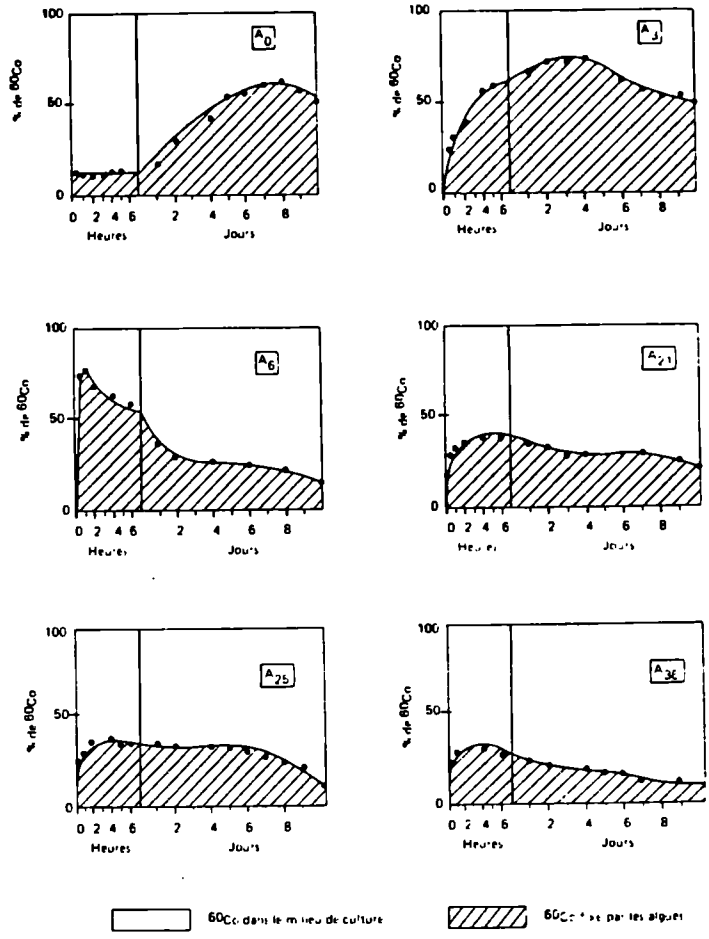
- Transfert du ^{60}Co vers une algue planctonique

Les expériences réalisées mettent en évidence une nette influence du mode de développement des cultures de Scenedesmus au moment de l'introduction du radiocobalt sur le degré de contamination de l'algue. Plusieurs hypothèses sont envisageables pour expliquer ce fait. Certaines reposent sur les modifications physiologiques au niveau des parois cellulaires consécutives au vieillissement. Il peut s'agir d'une variation dans la perméabilité membranaire faisant plus ou moins obstacle à la diffusion passive des éléments du milieu à l'intérieur des cellules. Ce peut être également une variation dans le nombre de cations échangeables des sites de surface ou dans l'attraction exercée par les charges électriques négatives de surface sur les cations du milieu. Parallèlement à ces phénomènes, compte tenu du caractère essentiel du cobalt au développement algal, on ne peut laisser de côté la participation d'un processus métabolique actif.

Les résultats obtenus suggèrent l'existence d'une relation entre l'activité des cultures et la participation respective de l'adsorption et de l'absorption dans la contamination de S. obliquus par le ^{60}Co.

Les valeurs élevées du facteur de concentration, qui peuvent atteindre 4.10^4 en fonction du poids sec, confirment le rôle particulièrement important que peut jouer le phytoplancton dans la fixation et le transfert du ^{60}Co dans les écosystèmes d'eau douce.

La décontamination des algues a été étudiée en renouvelant le milieu fréquemment. L'âge des cultures au moment de la contamination du milieu n'a pas d'influence significative sur la quantité de ^{60}Co éliminée par les cellules. La période biologique courte varie de 0,8h à 3 jours et la période biologique longue de 70 à 350 jours.



Accumulation du ^{60}Co par Scenedesmus obliquus en fonction de l'âge des cultures au moment de l'introduction du radioélément.
An = Age de la culture en jours.

- Transfert du ^{60}Co vers la carpe

L'étude engagée a pour but de comparer les cinétiques d'accumulation, les facteurs de transfert et les facteurs de rétention en fonction du type de nourriture. Les proies choisies, qui font partie du régime alimentaire de la carpe en milieu naturel sont lymnea stagualis, Gammanus pulex et des larves de chironomes.

Etude en Méditerranée

Le réseau des stations littorales marines encadrant le Rhône a été échantillonné, mensuellement, pendant deux ans.

Suite aux retombées de l'accident de Tchrnobyl l'étude des variations saisonnières d'activité chez quelques indicateurs biologiques en a été perturbée jusqu'à masquer, dans le cas de certains radionucléides comme le Rhodium 106, l'impact des rejets liquides des centrales. Cette perturbation est intervenue le plus nettement et à des niveaux équivalents, dans les stations du Sud Est du littoral français méditerranéen ainsi que dans celles proches de l'embourchure du Rhône. Dans ces deux zones, on a pu constater en 1987 un retour aux niveaux initiaux de l'activité de la plupart des radionucléides (césium 137 et rhodium106).

IV. Objectives for the next reporting period.

- Poursuivre les campagnes de prélèvements sur le Rhône et en Méditerranée et effectuer un bilan approfondi de l'impact de Tchernobyl comparé à celui des installations nucléaires sur le bassin Rhodanien. Evaluer la teneur en tritium des compartiments du fleuve en fonction des termes sources.
- Achever l'étude du transfert du ^{60}Co à une algue planctonique en prenant notamment en compte l'influence de la concentration du radionucléide et du cobalt stable ainsi que l'effet de la contamination chronique du milieu.
- Terminer l'étude comparée de l'accumulation du ^{60}Co par la carpe ingérant divers types de nourriture marquée.

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

- Département de Radiobiologie - CEN-SCK-MOL (prof. Kirchmann).
- Université Catholique de Louvain la Neuve (prof. Mittenaere)
- Département de Botanique de l'Université de Liège (prof. Sironval et Lambinon)
- Modélisation mathématique de l'Université de Liège (prof. Smitz)
- Faculté de Namur (prof. Descy).
- LNETI - Portugal (prof. Bettancourt).

Cette année nous avons établi une collaboration pour l'étude du Tage avec l'Université d'Estramadure en Espagne (prof. Conrado).

VI. Publications:

NUCHO R. & BAUDIN J.P. (1986). Données expérimentales sur la rétention du ^{60}Co par un algue planctonique, Scenedesmus obliquus. Influence de la température et de la photopériode. Sciences de l'eau, 5 (4) : 376.

LAMBRECHTS A., & FOULQUIER L, (1987). Radioecology of the Rhône Basin : Data on the Fish of the Rhône (1974-1984). J. Environ. Radioactivity, 5 (2) 105.121.

FOULQUIER L., LAMBRECHTS A., et PALLY M. (1987). Impact radioécologique d'une usine de retraitement de combustible nucléaire sur un fleuve : le Rhône. Proceedings of an International Conference on Nuclear Fuel Reprocessing and waste management. Paris, August 23-27, 1987. Société Française d'Energie Nucléaire : 1063-1071.

FOULQUIER L. (1987). Data concerning the radiocontamination of the freshwater ecosystems after the Tchernobyl accident. Seminar C.E.E./J.E.N. International Union of Radioecologists. IX th Annuel Meeting, Madrid, September 15-19, 1986, Extended Summaries of the Contributions, IUR Secretariat Ed., 20-24.

BAUDIN J.P. & FRITSCH A.F. (1987). Retention of ingested ^{60}Co by a freshwater fish. Water, Air, and Soil Pollution, **36** : 207-217.

NUCHO R., RAMBAUD A., FOULQUIER L. et BAUDIN J.P. (1988). Bioaccumulation du ^{60}Co par une algue planctonique, Scenedesmus obliquus Türps. (Kütz). Influence du stade de développement de la culture sur la fixation du radionucléide, (sous presse).

CALMET D., CHARMASSON. S, GONTIER. G, DABURON. L'impact des retombées de Tchernobyl sur Mytilus sp. prélevée sur le littoral français de la Manche et du bassin méditerranéen occidental. IV Symposium de radioécologie 14-18 Mars 1988 - Cadarache.

CALMET. D, GONTIER. G, CHARMASSON. S, BOUDOURESQUE. C.F., Spatio-temporal variation of radionuclides in the seagrass Posidonia océanica after Chernobyl radiation fallout. (à paraître).

Title of the project no : 4

Behavior of radionuclides in marine, freshwater and terrestrial environments
Radium transfer in freshwater ecosystems.
Experimental studies and fiels studies in the environment of french mining complex

Head(s) of project:

GRAUBY, A. et FOULQUIER, L.

Scientific staff:

DESCAMPS,B. - BAUDIN-JAULENT, Y. - REMILLET, 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 et de la production du combustible. Le programme est limité aux aspects liés à la radioécologie des eaux continentales. Le radium 226 est le radionucléide principalement étudié, mais on s'interesse aussi à l'uranium 238 et au plomb 210.

II. Objectives for the reporting period:

En 1987 deux aspects ont été abordés.

- L'étude expérimentale en laboratoire contrôlé, de l'accumulation et de l'élimination du radium 226 par la carpe (Cyprinus carpio).

Six expériences ont été réalisées : la contamination des carpes par voie directe (eau seule) et par voie trophique (par l'intermédiaire de crustacés) et leur décontamination après une contamination par voie directe. La répartition par organes a été étudiée à la fin de chacune de ces trois expériences.

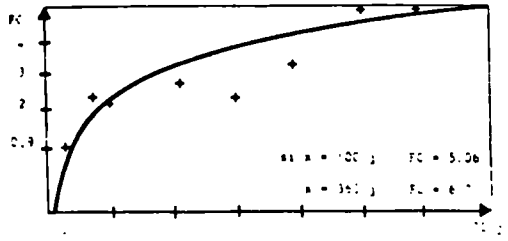
- Une étude de terrain par prélèvements d'eau, de sédiments, de végétaux et de poissons sur le site de stockage de résidus miniers du FOREZ. De plus une expérience in situ visant à démontrer la part prise par la voie directe dans la contamination globale a été réalisée sur ce site de stockage (expérience en "cages flottantes").

III Progress achieved

I - LES ETUDES EXPERIMENTALES EN LABORATOIRE CONTROLE

A -Contamination de la carpe par voie directe (eau seule).

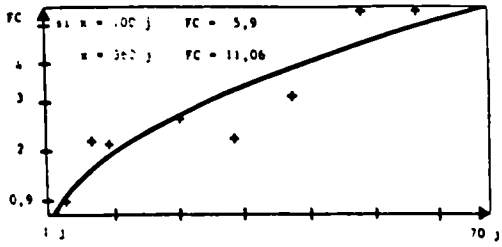
Le facteur de concentration (FC) obtenu en fonction du temps atteint une valeur de l'ordre de 5 après 60 jours. L'exploitation mathématique des résultats (courbes ci-dessous) prévoit un FC de 6,7 après un an d'expérience dans le cas d'une courbe logarithmique et d'un FC de 11 dans la cas d'une courbe de puissance.



Courbe logarithmique

$Y = -0,72 + 1,2 \log X$

$R = 0,84$



Courbe de puissance

$Y = 0,62\ X^{0,5}$

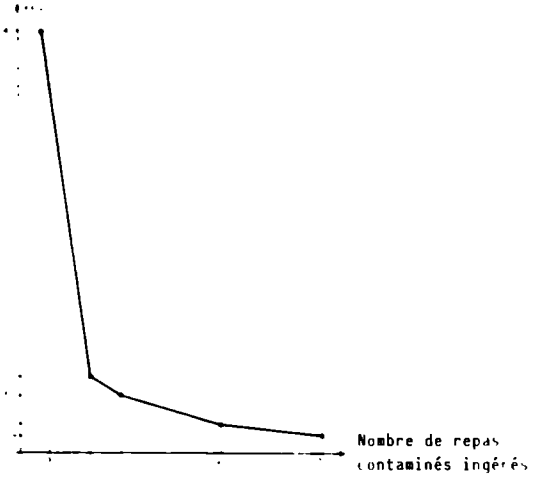
$R = 0,90$

Dans l'un et l'autre cas le FC par voie directe est faible par rapport au FC obtenu in situ : de l'ordre de 100 sur le site de Lodève et compris entre 170 et 1500 sur le site de stockage du FOREZ. Une expérience de contamination avec une eau peu chargée en calcium indique une faible influence de ce paramètre sur le FC.

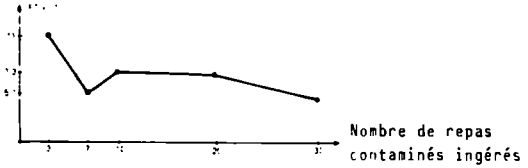
B. - Contamination par voie trophique

La contamination des carpes se faisant par l'ingestion de gammares (crustacés d'eau douce) préalablement contaminés on a suivi après 3, 7, 10, 20 et 30 repas l'évolution du facteur de rétention FR (rapport entre l'activité retenue par les carpes - en Bq - et l'activité ingérée - en Bq -) et celle du facteur de transfert FT (rapport entre la concentration - en $Bq.kg^{-1}$ frais - de l'organisme prédateur et celle de l'organisme proie - en $Bq.kg^{-1}$ frais).

Pour le facteur de rétention (courbe ci-contre) on enregistre une évolution très rapide en fonction du nombre de repas. Au delà de 20 repas il semble s'installer un régime d'équilibre autour de 3.10^{-3}, résultante des entrées et des sorties (excrétion du radium).

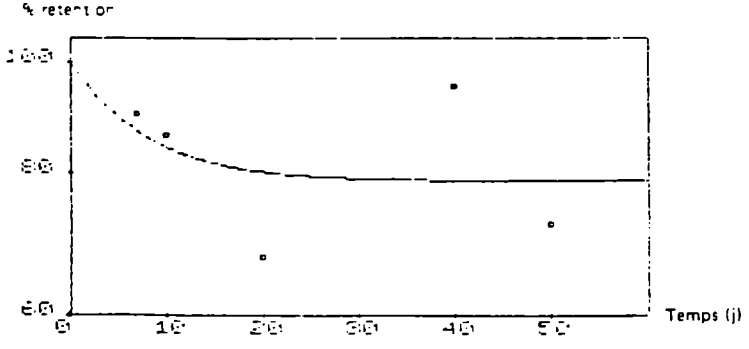


Pour le facteur de transfert (courbe ci-dessous) la valeur passe de 11.10^{-3} après 3 repas à 3.10^{-3} après 30 repas. Cette valeur laisse à penser qu'il ne peut y avoir bioamplification par cette seule voie de contamination.



C - Décontamination après une contamination par voie directe.

La décontamination a été réalisée après une contamination de 50 jours; elle a elle-même durée 50 jours. La courbe de décontamination ci-dessous, construite à partir du facteur de rétention exprimé en % (rapport entre la quantité de Ra restante et la quantité initiale) se caractérise par l'équation :



$$Y = 21\, e^{-0,137t} + 79\, e^{-10^{-3}\, t}$$

d'où l'on tire :

Tb1 = 5 jours, c'est la période biologique courte

Tb2 = 693 jours, c'est la période biologique longue.

On retiendra qu'au delà de 10 jours de décontamination, il reste environ 80% du radium qui s'éliminera avec une période biologique de l'ordre de 700 jours.

D - Répartition du radium dans les organes.

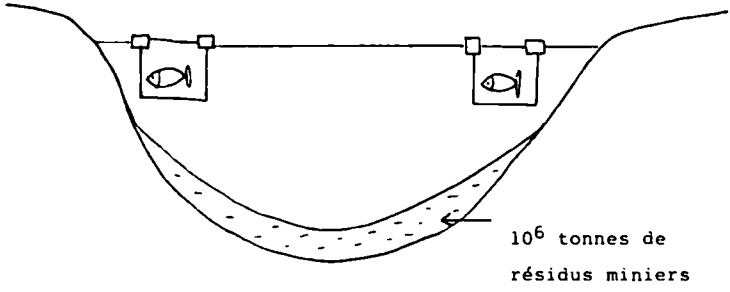
Citons les quatre conclusions principales.

- Les muscles, partie consommable du poisson, ne jouent pas un rôle important dans la physiologie du Radium ; ils ne représentent en effet qu'entre 4 et 9% de l'activité totale.
- Le squelette, contrairement à ce que l'on pouvait croire, n'est pas un organe particulier de fixation du Ra.
- Les branchies en contamination directe et le tube digestif en contamination par voie trophique ont des rôles importants.
- La peau semble avoir un rôle non négligeable durant la décontamination.

2 - ETUDES DE TERRAIN

Dans le lac (surface = 20 ha ; hauteur = 10 m) recouvrant les résidus miniers de l'ancien site du FOREZ nous avons introduit des cages flottantes contenant des carpes, des gardons, des tanches et des truites (voir le schéma ci-dessous). L'expérience a duré 2 mois pendant lesquels les poissons ont été nourris avec un aliment artificiel sous forme de granulé.

La concentration en radium de l'eau de ce lac est de l'ordre de 1 $Bq.l^{-1}$



IV. Objectives for the next reporting period:

1 - Etudes expérimentales en laboratoire contrôlé.
Etude de la contamination de l'algue phytoplanctonique Scenedesmus puis étude du transfert algue - daphnie ; la daphnie étant un petit crustacé d'eau douce entrant dans le régime alimentaire de la plupart des poissons.

2 - Etudes de terrain
Rédaction du rapport relatif aux 2 années de prélèvements sur le site du FOREZ et à l'expérience "cages flottantes". En fonction des résultats de cette expérience on pourra étudier la décontamination de poissons contaminés in situ.

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

- Section de Radioécologie Physique du SERE par l'intermédiaire de ces deux entités métrologie : le LMEI d'ORSAY et l'antenne de CADARACHE (M. PICAT, Chef de Section).
- L'antenne FOREZ du Département de Protection - SPIN pour la partie cages flottantes (M. ZETTWOOG, Chef du SPIN).

VI. Publications:

B. DESCAMPS et Y. BAUDIN-JAULENT - Etude radioécologique du complexe minier de Lodève (France) 1981-1985. Rapport CEA,R-5409, 98 pages.

B. DESCAMPS et L. FOULQUIER. Natural Radioactivity in the principal constituents of french river ecosystems. Fourth international symposium on Natural Radiation Environment. Lisboa, Portugal, December 7-11- 1987. 12 p.

Title of the project no.: 5

Cycling of tritium
Transfert de l'hydrogène tritié et du méthane tritié aux plantes. 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 de l'hydrogène tritié et des molécules tritiées les plus importantes, par les partie aériennes des plantes, en relation avec les paramètres climatiques et physiologiques et la composition de l'atmosphère. Le travail comporte des expériences de laboratoire et de terrain, ainsi que des tentatives de modélisation.

II. Objectives for the reporting period:

Au cours de l'année 1987, nous avons effectué des expériences de laboratoire pour étudier la captation du méthane et du formaldéhyde par la partie aérienne des plantes.

III. Progress achieved.

1) Etude en chambre expérimentale de la captation du méthane tritié par la partie aérienne des végétaux

Des expériences antérieures (Mason 1973) semblaient indiquer que l'incorporation du méthane tritié dans la matière organique des plantes était relativement importante. Pour vérifier et préciser ces résultats nous avons effectué des expériences en laboratoire pour étudier l'incorporation du méthane dans la partie aérienne des végétaux.

Les essais ont été effectués sur de plants individuels de tournesol âgés de quelques semaines et placés dans une chambre qui permet d'isoler la partie aérienne du végétal. Celle-ci était exposée à un flux d'air dont l'humidité relative est 90-95% et dont la concentration en méthane tritié était $2{,}2 \times 10^7$ Bq/m^3. Le méthane tritié utilisé était purifié par passage à travers une colonne de tamis moléculaire. Après 3 heures d'exposition, on mesurait le tritium dans l'eau libre des feuilles et l'eau de combustion de leur matière organique. 10 expériences ont été effectuées portant chacune sur une plante individuelle. La vitesse de dépôt sur les feuilles a toujours été inférieure à 10^{-6} cm/s. La valeur beaucoup plus grande trouvée par Mason pourrait être due à l'utilisation d'un méthane tritié imparfaitement purifié.

2) Etude en chambre expérimentale de la captation du formaldéhyde par la partie aérienne des végétaux

A l'occasion de travaux antérieurs nous avons pu observer que l'activité spécifique de l'eau de combustion de la matière organique des plantes était souvent beaucoup plus élevée que l'activité spécifique moyenne de leur eau libre. Cette observation nous a conduit à penser que le cycle du tritium était imparfaitement connu, et que les plantes pouvaient assimiler à partir de l'atmosphère des molécules tritiées autres que l'eau tritiée. Parmi les molécules candidates se trouvaient en bonne place l'hydrogène tritié et le méthane tritié qui sont à l'état de traces dans l'atmosphère, mais ont des activités spécifiques beaucoup plus élevées que la vapeur d'eau. Or, nous n'avons pu mettre en évidence aucune assimilation appréciable de l'hydrogène (travaux effectués en 1986), ou du méthane (travaux effectués en 1987 : voir ci-dessus). Restent quelque autres molécules simples dérivées du méthane, telles que le formaldéhyde, l'acide formique et le méthanol. De ces trois molécules le formaldéhyde est la plus abondante dans l'atmosphère. Compte tenu de sa réactivité, cette molécule pourrait servir de

véhicule pour le tritium et le carbone-14.
Des essais préliminaires d'incorporation du formaldéhyde dans les plantes ont été lancés en 1987, en utilisant, pour des raisons de disponibilité, du formaldéhyde marqué au carbone-14, étant entendu que le tritium lié à l'atome de carbone suit le même chemin que le carbone, au moins dans un premier temps.
Les essais ont été effectués sur des plants de tournesol, dont la partie aérienne a été exposée pendant 3 heures, à un flux d'air contenant 10^5 Bq/m^3 de formaldéhyde marqué, correspondant à une concentration de formaldéhyde d'environ 2 microgrammes par m^3 d'air. La vitesse de dépôt sur les feuilles, correspondant à l'incorporation dans la matière organique, a été déterminée sur 5 plants individuels. Elle atteint 0,2 cm/s pour des plantes ayant leurs stomates pleinement ouverts et diminue avec la conductance stomatique. Ces résultats préliminaires montrent que le formaldéhyde est capté de façon efficace par les plantes notamment par incorporation dans le matière organique. Des essais ultérieurs sont prévus pour préciser la cinétique de captation, déterminer les mécanismes en cause et évaluer l'importance de cette voie de transfert potentielle.

IV. Objectives for the next reporting period:

L'étude du transfert du formaldéhyde aux plantes et au sol sera poursuivie. On précisera sa cinétique du transfert et on essaiera de déterminer les processus chimiques ou métaboliques qui sont responsables de la fixation. Parallèlement on déterminera les concentrations de formaldéhyde tritié qui peuvent se trouver dans l'atmosphère, ou dans les effluents de diverses installations. Une évaluation sera faite de l'importance de cette voie de transfert du point de vue de la radioprotection.

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

VI. Publications:

Title of the project no.: 6

Impact des radionucleides relâchés en conditions accidentelles

Head(s) of project:

M. HUGON

Scientific staff:

M. HUGON

M. MAUBERT

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 Janvier 1987.

1/ - Mise au point d'un brûleur à haute température simulant une émission d'aérosols lors d'une fusion du coeur d'un réacteur nucléaire.

2/ - Dépôt des aérosols produits sur des végétaux.

3/ - Suivi des dépôts consécutifs à l'accident de Tchernobyl dans le Sud Est de la France.

III. Progress achieved:

Point 1 -

Le dispositif d'émission d'aérosols à haute température est shématisé figure 1.

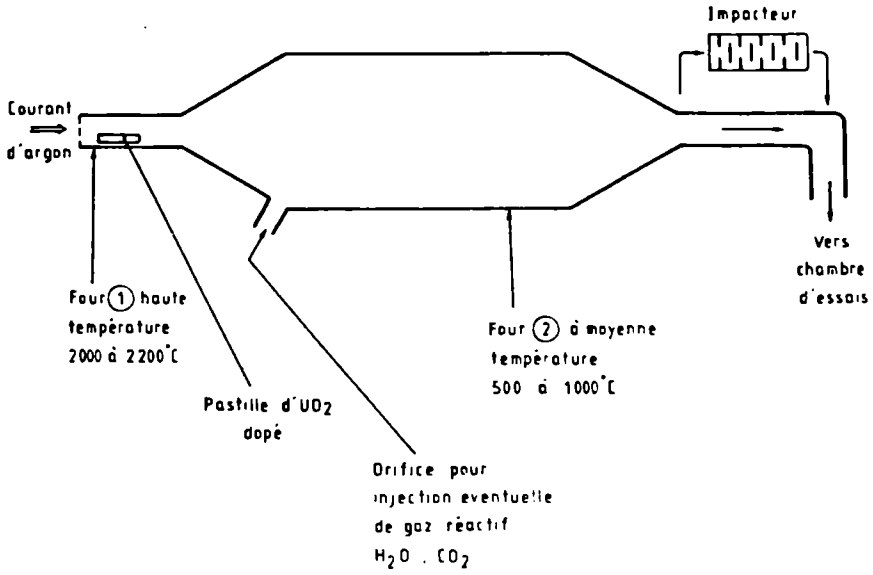


Figure 1 - Dispositif de production d'aérosols à haute température.

- Le four n° 1 est un four de graphite chauffé à l'électricité, la température peut atteindre 2200°C.

- La pastille destinée à simuler le combustible est fabriquée de la façon suivante :

du nitrate d'uranyle en solution est dopé à l'aide de ^{134}Cs sous forme carbonate, hydroxide ou iodure. Ensuite il y a évaporation et la poudre homogénéisée est réduite à 400°C sous courant d'hydrogène. Une compression permet alors de fabriquer une pastille de 4 mm de diamètre sous 2 à 3mm d'épaisseur.

Dans le four cette pastille est chauffée à 2200°C pendant 30 s sous courant d'argon, ce qui donne lieu à la libération d'aérosols, confirmée par le bilan des masses avant et après expérience.

- Un deuxième four (2) en quartz, ou la température peut varier de 500 à 1000°C et dans lequel on pourra injecter un gaz réactif permet de simuler les transformations chimiques éventuelles de l'aérosol produit dans l'enceinte de la centrale.

- A la sortie du deuxième four un impacteur permet de déterminer la granulométrie des aérosols (7 étages). L'essentiel des aérosols produits sont de tailles submicroniques. Sur ces filtres on peut déterminer la solubilité des produits appartenant à chacune des classes de granulométries.

En outre, des analyses par ESCA, (Electron Spectroscopy for Chemical Analysis) ont été réalisées. Il semblerait que les aérosols soient constitués de particules d'UO_2 autour desquelles une couche enrichie en césium soit agglutinée.

Point 2 -

Les aérosols sont envoyés dans une tente plastique de 2 m de haut sur 1,5 m de diamètre environ dans laquelle il est possible de disposer des végétaux. Pour l'instant l'expérience a été faite sur un Epicéa et sur des radis.

Des papiers filtres judicieusement disposés permettent d'effectuer des contrôles.

On a constaté que les vitesses de dépôt correspondaient à la loi de Stokes, soit 10^{-3} à 10^{-4} cm/s.

Dans la nature, les vitesses de dépôt habituellement rencontrée sont beaucoup plus fortes, de l'ordre de 1 cm . On envisage de modifier l'installation pour recréer la turbulence atmosphérique (ventilateur ?) afin d'augmenter les vitesses de dépôt.

Un rapport sur ces expérimentations est en cours de préparation et devrait être disponible vers le mois d'Avril 1988.

Point 3 -

Les travaux de terrain sur les dépôts consécutifs à l'accident de Tchernobyl se sont poursuivis. Ils ont donné lieu à une publication à Athènes en Octobre 1987. Un autre papier est en préparation. Il sera présenté au symposium de Cadarache en Mars 1988.

IV. Objectives for the next reporting period:

1/ - Poursuite de l'expérience de production d'aérosols à haute température. Amélioration du dispositif.

2/ - Poursuite de l'étude des dépôts consécutifs à l'accident de Tchernobyl.

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 - Belgique.

VI. Publications:

3/ - H. MAUBERT - A. GRAUBY - V. PONZETTO

Comparaison des prévisions de deux modèles radioécologiques avec des mesures réelles. Athènes Octobre 1987.

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 facteurs de transfert sol-plante pour le césium, le plutonium et l'américium issus des retombées atmosphériques. Cette étude est réalisée à partir de 8 parcelles cultivées en luzerne et ray-grass, implantées dans 4 régions du territoire français afin d'évaluer l'influence de situations géographiques et climatiques différentes sur le dépôt des radionucléides sur le sol et la végétation.

2/ - L'étude expérimentale du transfert sol-plante du neptunium et de l'américium en fonction des caractéristiques physicochimiques des sols et des types de végétaux. L'importance de ces transferts fait l'objet d'un suivi dans le temps, de même que l'évolution de la biodisponibilité des différents radioéléments dans les sols.

II. Objectives for the reporting period

1/ - Mise en place de la totalité des parcelles expérimentales et des cultures de luzerne et ray-grass.

2/ - Prélèvements périodiques d'échantillons de végétaux, de sol et d'eau de pluie sur ces parcelles.

3/ - Analyses des différents radioéléments contenus dans ces échantillons.

4/ - Réalisation d'analyses radiochimiques comparatives entre le CEA Cadarache et les laboratoires d'Harwell avec qui ce programme est mené en collaboration.

III. Progress achieved:

Au début de l'année 1987 les deux parcelles expérimentales situées dans le sud de la France, dont les cultures avaient subit des dommages à cause de la sècheresse de l'été 1986, ont été réensemencées en luzerne et ray-grass.

A partir du mois d'Avril jusqu'au mois de Novembre des récoltes mensuelles de végétaux ont été effectuées sur chacune des 8 parcelles. Ces prélèvements ont été groupés de façon à réaliser un échantillon composite par semestre et par site. Sur cet échantillon sont dosés le césium 137 (spectrométrie GeLi), les plutonium 238, 239 et 240 et l'américium 241 (spectrométrie α). Ces mêmes radioéléments sont mesurés dans les eaux de pluie collectées pendant les périodes correspondant aux récoltes des végétaux. L'exploitation de ces analyses permet de déterminer la radioactivité qui se dépose au sol ainsi que celle interceptée par les organes aériens des végétaux.

A l'heure actuelle, seules les analyses concernant le césium pour la totalité des échantillons du premier semestre 1987 ont été exécutées. Les résultats obtenus ont permis d'évaluer les dépôts au sol pour ce radioélément : de 10 à 71 Bq/m^2 suivant les sites. Quant aux végétaux les concentrations en césium varient de 2 à 35 Bq/au kilogramme de matière sèche. Les analyses des éléments transuraniens sont actuellement en cours.

Au mois de Décembre 1987, lors de la dernière récolte des végétaux, un échantillonnage de sol a été réalisé pour chaque site, afin de déterminer la répartition des radioéléments en fonction de la profondeur et d'évaluer les facteurs de transfert sol-plante.

En ce qui concerne l'étude expérimentale de l'influence des paramètres du sol sur l'absorption racinaire des transuraniens par les végétaux, trois types de sol (acide, calcaire, organique) contaminés par du neptunium et de l'américium ont servi de support à des cultures de salade, haricot et radis.

Le calcul des facteurs de transfert a montré que c'est sur le sol acide que l'absorption racinaire était la plus importante tandis qu'elle était la plus faible sur le sol organique et ce pour les trois types de végétaux.

IV. Objectives for the next reporting period:

Poursuite de l'échantillonnage mensuel sur l'ensemble des parcelles expérimentales. Réalisation des analyses des Cs, Pu et Am sur les végétaux, eaux et sols ainsi prélevés.

Echange périodique d'échantillons entre le laboratoire de Cadarache et celui d'Harwell, afin d'effectuer des intercalibrations.

Pour la partie expérimentale étude de l'évolution de la biodisponibilité des radioéléments contenus dans les trois types de sol utilisés.

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

Centre de recherches de Harwell (Environmental and Medical Sciences Division)
Chef : Dr. D. H. PIERSON.
Notre collaboration a lieu avec Monsieur P.A. CAWSE.

VI. Publications:

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 quatre objectifs :

1/ - Détermination des facteurs de transfert aux végétaux du césium et du cobalt dans le cas d'une contamination chronique du sol et dans le cas d'une contamination accidentelle touchant le sol ou la végétation.

2/ - Détermination des possibilités de réduire les transferts résultant d'une contamination accidentelle en utilisant des techniques culturales particulières : labour superficiel, labour normal ou labour profond.

3/ - Détermination des transferts pour des dépôts de radioéléments survenant à différents stades de développement des végétaux.

4/ - 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:

Au cours des années précédentes deux thèmes de recherche ont été abordés :

1/ - Etude du transfert sol-plante du césium 137 et du cobalt 60 pour une contamination homogène de la zone de labour du sol (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 radioéléments sur la surface du sol ou sur les parties aériennes des plantes.

III. Progress achieved.

En 1986 et 1987 une simulation de contamination accidentelle a été réalisée en pulvérisant sous forme d'aérosols des solutions aqueuses de césium 137 et de cobalt 57 sur des cultures de blé et de carottes. Les activités mises en oeuvre pour chacun des deux radionucléides ont été équivalentes à des dépôts de 3.10^7 becquerels par m^2 comme dans le cas de l'étude des transferts racinaires réalisée précédemment. L'apport de ces radioisotopes a été effectué selon les modalités suivantes :

1/ - D'une part uniquement sur les parties aériennes des végétaux :

- soit en une seule fois pour les cultures de 4 lysimètres,
- soit fractionné en trois fois au cours du cycle végétatif pour 4 lysimètres.

2/ - D'autre part uniquement à la surface du sol :

- soit en une seule fois pour 4 lysimètres,
- soit fractionné en trois fois au cours du cycle végétatif pour 4 lysimètres.

Les concentrations moyennes en césium et cobalt mesurées dans les grains de blé ont été les suivantes, en fonction du mode de contamination :

1/ - Transfert dû à l'absorption racinaire résultant de la contamination homogène des 30 premiers centimètres du sol :

Césium 137 = 1,2 ± 0,5 Bq par gramme de grain

cobalt 60 = 0,8 ± 0,3 Bq par gramme de grain

2/ - Transfert dû à la contamination accidentelle déposée à la surface du sol ou sur les organes aériens des végétaux :

- Apport unique à la surface du sol :

Césium 134 = 8 ± 0,5 Bq par gramme de grain

cobalt 57 = 1,8 ± 0,7 Bq par gramme de grain

- Apport fractionné à la surface du sol :

Césium 134 = 7 ± 0,6 Bq par gramme de grain

Cobalt 57 = 1,4 ± 0,3 Bq par gramme de grain

- Apport unique sur les plantes :

Césium 134 = 500 ± 50 Bq par gramme de grain

Cobalt 57 = 325 ± 42 Bq par gramme de grain

- Apport fractionné sur les plantes :

Césium 134 = 475 ± 45 Bq par gramme de grain

Cobalt 57 = 340 ± 30 Bq par gramme de grain

La première étape de cette étude avait conduit en 1985 et 1986 à la détermination des facteurs de transfert sol-plante du césium 137 et du cobalt 60 pour des cultures de céréales (blé, orge) et pour une plante maraîchère (salade) dans le cas d'une contamination répartie de façon homogène dans les 30 premiers centimètres du sol de 16 lysimètres. Ce type de contamination correspond à une situation résultant du dépôt au sol des radionucléides issus des rejets chroniques d'installations nucléaires durant une longue période de temps et qui, sous l'action des pratiques culturales, se trouvent en permanence incorporés à la zone labourée du sol. Dans le cas de notre expérimentation la contamination apportée correspond à un dépôt au sol de 3.10^7 Becquerels par m^2 de césium 137 et de cobalt 60.

En outre, cette expérimentation a permis de montrer que l'apport au sol de cobalt et de césium stables, à des niveaux de concentration de 50 et 10 ppm respectivement pour chacun des deux éléments, provoquait une augmentation de l'absorption racinaire de l'isotope radioactif correspondant. Ces résultats sont consignés dans le tableau suivant qui indique la valeur des facteurs de transfert sol-plante calculés par rapport à la matière sèche pour chacun des végétaux expérimentés.

		Blé		Orge		Salade
		Paille	Grain	Paille	Grain	Feuilles
Facteur de transfert pour le cobalt	sol + cobalt stable	7,3 E-2	3 E-2	6,9 E-2	2,9 E-2	1,2 E-1
	Sol sans cobalt stable	9,6 E-3	7,2 E-3	9,4 E-3	7,3 E-3	6,5 E-2
Facteur de transfert pour le césium	sol + césium stable	8,8 E-2	6,1 E-2	9 E-2	6,4 E-2	3,1 E-1
	sol sans césium stable	7,3 E-3	4,2 E-3	7,1 E-3	4 E-3	5,4 E-2

Ces résultats permettent de tirer les conclusions suivantes :

- D'une part, que les transferts racinaires sont sensiblement plus importants s'ils résultent d'un apport des radioéléments à la surface du sol par rapport à ceux issus d'une contamination équivalente mais incorporée de façon homogène dans la zone d'exploration des racines,

D'autre part que le transfert par voie foliaire est supérieur de 1 à 2 ordres de grandeur au transfert par voie racinaire.

- Enfin que, dans les conditions de cette expérience, le fractionnement de l'apport des radionucléides sur le sol ou sur la végétation n'a pas modifié de façon significative le niveau de contamination du produit récolté par rapport aux résultats d'un apport unique.

Les premières valeurs des analyses effectuées sur les cultures de carottes indiquent une tendance similaire. Cependant tous les résultats ne sont pas disponibles à l'heure actuelle du fait que la récolte a eu lieu au mois de novembre 1987.

A la fin de l'année 1987 nous avons également fait subir divers types de labour aux sols des lysimètres ayant reçu le dépôt "accidentel" de radioéléments en surface. La couche superficielle contaminée a été, pour certains, disposée à 30 cm de profondeur (labour normal), tandis que pour d'autres, elle a été placée à 50 cm de la surface (labour profond).

IV. Objectives for the next reporting period

Etude de l'influence des diverses pratiques culturales (labour superficiel, normal ou profond) sur les niveaux des transferts sol-plante pour différentes cultures: blé, salade et luzerne.

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 F. FURH, W. STEFFENS et W. MITTELSTAEDT.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor.

Contract no : BI6-B-038-UK

Natural Environment
Research Council
Polaris House
North Star Avenue
GB- Swindon SN2 1EU Wilts

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
Institute for Marine Environmental Research
Prospect Place, The Hoe,
Plymouth, Devon PL1 3DH, U.K.

Scientific staff:

i Mr. R.J. Clifton
ii Assistant Scientific Officer services

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:

To determine whether or not organic or inorganic surfaces are more important for the uptake of radionuclides. To validate an alpha particle (^{242}Cm) back-scatter analyser for the determination of selected elements on the surface of materials. To consider analytical methods likely to provide real environmental data, rather than those which include significant artifacts related to methodology.

III. Progress achieved:

1. Methodology.-A number of techniques have been developed in order to examine radionuclide-particle interactions using natural systems on small amounts of samples. Electrochemical methods are now being used to study "Speciation" and complexation of radionuclides at specific sites. New approaches to determining the distribution of alpha particle emitters are being validated for small samples. The backscatter analyser continues to provide supportive information concerning the overall composition of surfaces, especially C,N,O and Fe, see Fig 1.
2. Results.- Using capacitance of seawater (polarography HMD) as an index of relative water quality for a transect - NE Irish Sea - SW Approaches-English Channel - S North Sea; this correlates with an ability to determine U in seawater by catechol, or oxine, which in turn reflects complexation capacity of the water. Using U as a model system the approach is being extended to Pu and Am. Waters off Cumbria have enhanced complexation capacity, probably derived from an influx of polluted Liverpool Bay water moving north. A number of compounds related to complexation are being examined. The migration of complexing agents to the sea surface and enrichment at the surface is high. The concentration and isotopic composition of U in seawater and particulates (Irish Sea to N. Sea) has been determined (n=30) and they correlate with water quality and source regions.

So far a large number of physical separations have failed to alter some 10 different radionuclides ratios for Esk and other sediments, hence they may be associated with "one matrix". Interest centres upon the properties of natural magnetite (1-5% vol. sediments) using cryogenic, electromagnetic and permanent magnet separations. The SIROFLOC process is being examined on natural systems together with coated magnetite grains. The study also includes disseminated natural biogenic magnetite. The cohesive structure of fine grained Esk and Irish Sea sediments is being examined in terms of any effect attributable to aggregates of magnetic particles. Multi-element study of the geochemistry of the Esk and Irish Sea sediments has been completed and will be interpreted in relation to source regions. The isotopic composition of stable lead (207/206Pb and 208/204Pb) for Esk and Irish Sea sediments is different in the <63 and >63μm fractions which is not accounted for by the distribution of stable detrital Pb. A probable origin is from Liverpool Bay contaminants; the lead isochron indicates Hercynian materials for the sediments of the Irish Sea and Caledonian for the Esk. On the basis that the activity of hot particles in Esk sediments arises from U +Pu, Am +gamma emitters, they probably represent spent fuel debris from BNF cooling ponds. The activity of these particles has been compared with natural hot particles for the UK.

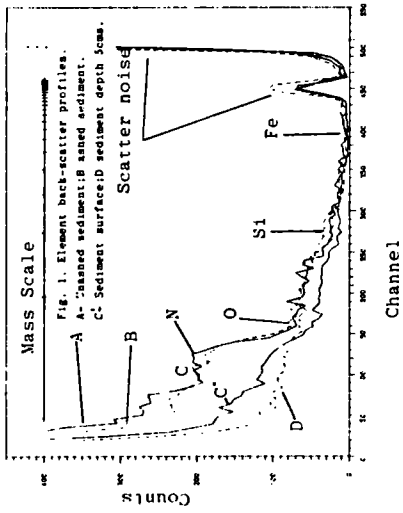
Data are being obtained concerning the selective loss of short lived alpha emitters, eg for 234-U, 238-Pu, 228-Th and 224-Ra, as a consequence of recoil or selective leaching from radiation damaged regions.

Uptake and bioavailability of a prime surface seeker namely 210-Po has been determined for Esk mussels; the order of activity is kidney>gonads> digestive gland>total soft> byssus>foot>muscle; relatively high levels in stable gonads are unusual. The origin of the Po, together with large amounts of U, 226-Ra relative to BNF output is from a phosphate fertiliser plant (see Hamilton E.I. Ind. Hyg. Assn J. 32, 398-403, 1971). The coastal region near St. Bees Head contains some of the highest 210Po values found so far in the UK. Oceanic studies continue in order to trace the total pathway of radionuclides on surfaces from the land to the deep ocean. The use of in-situ high volume (20001) seawater filtration has been validated for 234Th studies. New approaches to examine total and

specific alpha emitters in small (eg 2 litres) volumes of seawater with high spatial resolution over the range, surface to 4000m depth has been validated. In one cruise the method identified Mediterranean water at depth off Portugal. In the Porcupine Sea Bight the presence of higher alpha active material associated with lateral fluxes of nephloid material has been identified as illustrated in Fig 2. In the open ocean the importance of uptake of radionuclides by biota in relation to productivity has been examined. All these studies are backed up by a comprehensive examination of biota, faecal debris, C,N, salinity and temperature data.

In relation to aerial deposition onto surfaces limited research continues with Chernobyl debris. Using deposition on Usnia 137-Cs in SW England is decreasing with an overall retention of 0.5y. However, for the first time 241-Am is possibly detected in Usnia. In examining the penetration of Chernobyl debris in a lake sediment and ombotrophic peat (from Devon) the former shows a high 137-Cs peak at 15cm and the latter at 9cm from the surface; there has been no penetration of 234-Th or 7-Be, while natural emitters show an expected distribution; 241-Am is also present at the peak activities. The origin of the activity is being considered further.

3. Discussion.- The reserach continues to be placed on a broad basis, especially geography, but attention is focussed throughout on mechanisms of uptake and loss of radionuclides by surfaces (biotic and abiotic) through major ecosystems; more attention is now being directed towards the alpha active heavy elements. As some of our major instrumentation fails because of extreme age, where possible the research will continue through use of innovative simple methods, several of which show high promise.



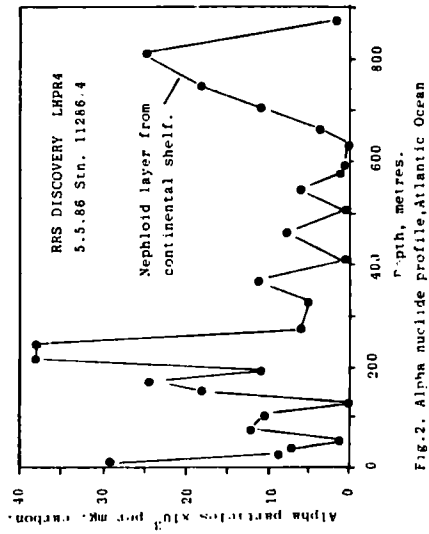


Fig.2. Alpha nuclide profile,Atlantic Ocean

IV. Objectives for the next 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 ferromagnesium 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 debis.

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

During 1987 the nature of the research was not conducive to any substantial collaboration with others. Requirements for 1988 are only now being assessed, but cooperation will continue with MAFF Radiobiological Laboratory, Lowestoft and the Deacon Laboratory NERC, Godalming, the former in relation to the Irish Sea and UK coastal waters and both in relation to the Atlantic Ocean. Within the restriction of available funds other types of cooperation may be considered when appropriate.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no : BI6-B-044-UK

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

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

Dr J.S. Hislop
Env.& Med. Sciences Div.
Harwell Laboratory
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 estimation of the total deposit of actinides in the estuary.

Head(s) of project:

Dr J S Hislop,

Environmental and Medical Sciences Division,

Harwell Laboratory, Didcot, Oxon OX11 ORA, UK

Scientific staff:

Dr R Carpenter

Mr P J Burton

Miss L P Yarnold

I. Objectives of the project:

To extend and confirm the previous observations that a) there is a net loss of actinides from the Ravenglass estuary in each tidal cycle and b) that this loss is due mainly to actinides adsorbed on suspended sediment. In addition, in order to establish what proportion of the total actinide inventory is lost during each tidal cycle, an estimate of the total inventory is being made. This involves taking a large number of sediment cores from the estuary for actinide analysis. By relating this to the amount lost with each tide, it will be possible to calculate the residence time of the deposit.

II. Objectives for the reporting period:

It was intended that during the present reporting period the analysis of bulked core samples from the estuary would be completed and the final estimate of the actinide inventory of the estuary would be calculated. A further tidal balance study in the estuary in storm conditions was planned. This would involve development of remote sampling techniques.

In addition, a report on all the research carried out by this group in the Ravenglass estuary, under (CEL and other funding, was to be prepared for publication in the open literature.

III. Progress achieved:

Methodology: Actinide analysis of the 38 bulked core samples has been completed.

An experiment was designed to take mid-stream, mid-depth samples remotely from the shore in weather conditions too stormy to permit working from moored boat. This involved a series of sampling pipes attached at different distances below a floating buoy in order to sample from mid-depth at all states of the tide. Samples were pumped the 150m to the shore and then filtered through 0.22 μm Millipore filters.

Two tides were sampled in this manner in the autumn of 1987. On the first occasion, despite favourable weather forecasts, conditions were quite calm. The second experiment was carried out in somewhat rougher conditions, but they were not as stormy as had been hoped. Samples from the first experiment have been bulked in proportion to the water flow and volume in order to give gross values of actinides in solution and suspension on the flood and ebb tides. This will reduce analytical costs significantly, whilst still providing a data set of activity balances. The samples from the second experiment will be analysed individually to give greater detail on K_d values and specific activities along with the activity balance data.

Results: Typical levels of $^{239+240}Pu$ deposited in the estuary range from ~20 Bq kg^{-1} (dry weight) in sandy areas to ~5kBq kg^{-1} in the saltmarshes. Table I gives a summary of the integrated deposits of $^{239+240}Pu$, ^{238}Pu and ^{241}Am in the estuary at the time of sampling (1985-6), without allowance for further ingrowth of ^{241}Am from ^{241}Pu in the sediments.

Analysis of the samples from the two tidal cycles is not complete but the work is on schedule to meet the draft and final reporting dates for the project of 30/8/88 and 30/11/88 respectively.

Discussion: The inventories quoted in Table I are lower than was predicted from a single saltmarsh core from the Esk discussed in the 1985 progress report on this project. Provisional estimates of ~2000 GBq each of $^{239+240}Pu$ and ^{241}Am were made at that time based on some

assumptions which have proved to be inaccurate. It was thought that the average activity in the Mite and Irt saltmarshes would be approximately equal to that in the Esk, and the total inventory of the silt and sand areas was expected to be similar to that of the saltmarshes. In fact the levels in the Esk saltmarshes proved to be a factor of 2-3 higher than those in the other rivers and, as can be seen from Table I, the activities in the other deposits are much lower than in the saltmarshes, despite representing about 50% of the total area of the estuary.

Area	$^{239+240}Pu$ GBq	^{238}Pu GBq	^{241}Am GBq
River Esk Saltmarshes	177.1	37.7	227.0
Remainder of River Esk	40.8	7.9	83.3
River Mite	50.0	10.4	66.4
River Irt Saltmarshes	60.6	11.7	72.0
Remainder of River Irt	45.9	9.4	58.4
Estuary Mouth	3.0	0.6	6.8
Total	377.4	77.7	513.9

Table 1: Total activity in sediment deposits of the Ravenglass estuary

IV. Objectives for the next reporting period:

This contract terminates in the next period. The objectives for the period are completion of all outstanding analysis on the samples from the two tidal cycle experiments of autumn 1987 and preparation of the final report on all the work carried out by this group in the Ravenglass estuary.

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

None.

VI. Publications:

(1) Burton, P.J. Laboratory studies on the remobilisation of actinides from the sedidments in the Ravenglass estuary. Sci. total Environ., 52, (1986) 123-145.

(2) Eakins, J.D., Burton, P.J., Humphreys, D.G. and Lally, A.E. The remobilisation of actinides from contaminated intertidal sediments in the Ravenglass estuary. Proc. Seminar on The Behaviour of Radionuclides in Estuaries, Renesse, Netherlands, 1984, CEC Seminar XIII/380/85-EN (1985).

(3) Burton, P.J., Eakins, J.D. and Lally, A.E. The significance of distribution coefficients in the remobilisation of actinides from intertidal sedidments of the Ravenglass estuary. Proc. Seminar on the Application of Distribution Coefficients to Radiological Assessment Models, Louvain-la-Neuve, Belgium, 1985, (1986).

(4) Burton, P.J. and Yarnold, L.P. Remobilisation of Actinides from intertidal sediments of the Ravenglass estuary. Proc. 5th RSC Seminar on Environmental Radiochemical Analysis. Harwell, UK, 1986 (in press).

Title of the project no.:

(2) The source of actinide-bearing sedidments in the surf zone in West Cumbria.

Head(s) of project:

Dr J S Hislop, Environmental and Medical Sciences Division,
Harwell Laboratory, Didcot, Oxon OX11 ORA, UK

Scientific staff:
Mr P J Burton

Miss L P Yarnold

Dr R C Carpenter

I. Objectives of the project:

In West Cumbria actinides are transported to the land by sea spray (1). This spray is enriched with actinides relative to their concentration in bulk seawater due to sediment in the surf zone being concentrated by foam flotation and bubble bursting. The source of actinide-bearing sediment in the surf zone is unknown and the aim of this project is to investigate the hypothesis that it may be the mud bank in the Irish sea off St Bees and possibly include the sediment deposits in the Ravenglass and Duddon estuaries and the Solway Firth.

II. Objectives for the reporting period:

It was proposed that during this reporting period the sea spray particulate samples collected in 1985 and 1986 and the sediments from the estuaries and the offshore mud bank would be analysed by the sequential extraction technique of Tessier et al (1). It was hoped that the results obtained would enable conclusions to be drawn about the source of actinide-bearing sediment in sea spray and an explanation to be made for the seasonal variation in enrichment factors for actinides in sea spray in W. Cumbria.

III. Progress achieved:

Methodology: 1g aliquots (dry weight) of sea spray sediments, surf zone suspended material, estuarine and offshore sediments were subjected to the Tessier extraction scheme (1) in which trace elements are partitioned into five fractions (Tables I and II). The fractions obtained were analysed for actinides.

Results: Selected data for $^{239+240}Pu$ are given in Tables I and II. Values for sea spray and suspended particulate at Drigg were similar to the other sites and the Solway Firth was like the Duddon Estuary. ^{241}Am showed the same trends as plutonium, but with greater association with carbonate and less with the residual phase.

Discussion: It is dangerous to over-interpret these data and only general trends should be taken as both counting statistics and slight variations in extraction conditions can introduce errors.

The activity collected on a muslin screen depends on parameters such as site, exposure time, wind speed and sea conditions. Thus, in comparing results, percentages, rather than absolute values, should be used.

There is more Pu in the organic fraction of sea spray particulate in the autumn than the spring. This could be explained by uptake by growing microorganisms, which when they decay in autumn, leave fine particulates, with organically-bound Pu, in suspension and available for scavenging by rising bubbles in the surf zone and injection into the air as spray. This mechanism could explain the autumn peak in actinide concentrations in air observed consistently at Eskmeals in 1978-86.

Particulate material in the surf zone and in sea spray at Eskmeals has a similar distribution to that found in the Ravenglass estuary. Actinides are lost from the estuary on the ebb tide in association with suspended material which is transported into the nearby intertidal region to form part of the particulate content of sea spray. In this region only 0.1% by weight of the intertidal deposits are <10 μm in size, so fine material in sea spray must be continually brought into the surf zone from outside, rather than coming from the action of waves on the beach. The present study indicates that the Ravenglass estuary, and other areas of muddy deposits such as estuaries and the offshore mudbank may be the source of

some of the actinide-bearing fine sediments in sea spray at nearby beaches. If this is the case, actinide levels in sea spray will decrease only slowly as contaminated deposits disperse.

Fraction	Activity, Bq Kg^{-1}(dry weight); (% of total)			
	Eskmeals		St Bees/Seamills	
	Spring	Autumn	Spring	Autumn
Exchangeable	2(0.2)	1(0.1)	2(0.2)	2(0.1)
Carbonate	27(2.5)	30(2.5)	14(1.8)	36(1.0)
Fe/Mn oxides	403(37.1)	315(26.7)	225(28.5)	844(23.4)
Organic	214(19.7)	370(31.4)	109(13.8)	693(19.3)
Residual	441(40.5)	463(39.3)	442(55.7)	2028(56.2)
Totals	1087(100)	1179(100)	792(100)	3603(100)

Table 1: Distribution of $^{239+240}Pu$ in sea spray particulate

Fraction	Activity, Bq Kg^{-1}(dry weight); (% of total)			
	Surf zone sediment	Surface sediment		
	Seamills	Ravenglass Estuary	Duddon Estuary	Offshore Mudbank
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 2: Distribution of $^{239+240}Pu$ in surf zone suspended sediment and surface deposits

IV Objectives for the next reporting period:

This project terminates in the next period. No outstanding experimental work remains, but it is felt that more detailed studies of the phenomena discussed are required before a literature report can be written. Accordingly funding is being sought from UK Government Departments to continue studies into other aspects of this work such as variabilities in enrichment with distance from the source, seasonal differences and factors affecting sea spray generation and sea-to-land transfer of actinides in West Cumbria.

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

None.

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 impotance, 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:

For brackish waters, two of the main chemical and physical determinants are pH and salinity. To enhance our understanding about their influence upon the bioavailability of ^{241}Am and its speciation, we studied the uptake of ^{241}Am by the amphipod Gammarus duebeni duebeni, in combination with filtering experiments, varying these conditions.

Feeding experiments should complete our information about the uptake and accumulation of ^{241}Am, by the gastro-intestinal tract of Gammarus duebeni duebeni, from contaminated particles.

III. Progress achieved:

1. Methodology

All animal tests were made as batch experiments using adult males of the euryhaline amphipod Gammarus duebeni duebeni. For the investigations of ^{241}Am uptake from solution, water in 4 salinity/pH combinations were used: S 10/pH 7 to 8, S 10/pH 6 to 7, S 1.1/pH 7 to 8, and S 1.1/pH 6 to 7, and initial concentration of 0.6×10^{-9} mol x dm^{-3} of ^{241}Am. 10 ml of the batch solutions were filtered through 0.01 um pore size cellulose nitrate filters. Feeding experiments were made using pieces of algae contaminated in a ^{241}Am solution.

2. Results

Uptake of ^{241}Am by gammarids at selected salinity/pH ranges reveals only small differences. At S 10/pH 7 to 8, S 10/pH 6 to 7, and S 1.1/pH 7 to 8 Am uptake can be described by one-term exponentials, the plateaus reached having nearly the same level. Uptake from S 1.1/pH 6 to 7 follows the course of a two-term exponential, increasing slightly after a sharp initial rise. More essential differences in uptake behaviour can be found in the initial increases during the first week, which steepen from S 10/pH 7 to 8 over S 10/pH 6 to 7, S 1.1/pH 7 to 8 to S 1.1/pH 6 to 7.

During moults, animals lose 40% to 80% of their body-burden. Initial uptake of Am after moulting during the first week is higher than that of animals which have not moulted. Extremely high body-burden was achieved by 1 speciman, which was exposed at S 1.1 and pH 6 to 7 just after moulting. If a newly shed cuticule was removed from the beaker, the pH of the batch water becomes much lower than usual on the next day.

When determining Am decrease in the seawater batches before contact with animals, the loss of ^{241}Am to the walls of experimental units increases with S 1.1/pH 7 to 8 over S 10/pH 7 to 8, S 1.1/pH 6 to 7 to S 10/pH 6 to 7. When filtering these spiked waters, the part of filterable ^{241}Am decreases from S 10/pH 6 to 7 over S 10/pH 7 to 8, S 1.1/pH 6 to 7 to S 1.1/pH 7 to 8. After contact to animal material (and therefore humic), the amount of filterable Am in the seawater solutions continues to diminish.

In feeding experiments, after single delivery of contaminated algae, the body burden decreases hyperbolically to a very low persistent

value. Feedings of non-cantaminated algae during the loss phase result in a stepwise diminuation of the body-burden by excretion shortly after feeding. Repeated feeding with contaminated algae leads to a slight increase of the low persistent value. Sporadic moulting during such experiments reveals that a very small part of the entire ^{241}Am body-burden is associated with the exoskeleton.

3. Discussion

Accumalation of ^{241}Am in gammarids by uptake from solution depends upon the pH and salinity of the seawater: it is higher at low pH than at high pH and higher at low salinity than at high. Variations in salinity affect the bioavailability to a larger extent than changes in pH. At S 1.1 and pH 6 to 7, uptake by gammarids seems to liberate ^{241}Am bound to container walls and particles. Moulting significantly influences the uptake of ^{241}Am during the first week after adding the nuclide to the solution. Shortly after beginning of the experiment, less ^{241}Am might be bound to humic material, which could result in a higher bioavailability. The decrease of pH in the seawater batches after moulting, as well as the rare moults of the animals kept in seawater of 1.1 are assumed to be caused by the limited concentrations of Ca^{2+} and CO_3^{2-} in the batches. The results of the filtering experiments are remarkable: Low salinity filtrates contain less ^{241}Am than those of high ones, contrary to what one would expect from the results of the uptake experiments. If this effect is not due to the experimental design (matrix effect), it could be caused by competition, probably with Ca^{2+} ions.

Experiments with contaminated food lead to a very small persistent accumulation. At this state of the investigation it cannot yet be decided (a) if this accumulation is due to uptake from food via the gastro-intestinal tract, or to uptake from material released into the water by the algae or excremental pellets; and (b) if an incomplete digestion or simple passage through the gastro-intestinal tract is responsible for the high loss after feeding with algae.

IV. Objectives for the next reporting period:

In order to obtain more information about competition of Ca(2+ and Am-241, the experiments will be supplemented by vaying the Ca(2+) content of the experimental solutions. By changing the spiked waters every day, an attempt will be made to lower the increase of humic substances in the seawater batches. Investigations about speciation will be carried on by more differentiated filtration/ultrafiltration of the waters used. Furthermore, the mixing zones of estuaries are to be simulated experimentally by the addition of metallic hydroxides, silicic acids, complexing agents, and suspended particulate matter. Then it is intended to take water from the estuary of the River Elbe for our experiments.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Contract no : B16-B-040-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)]:

Ir. R. Kirchmann
Département de Radio-
biologie, CEN/SCK
Boeretang 200
B-2400 Mol

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:

M. Hegela, R. Kirchmann, M. Lambiet-Collier
J. Maisin, C. Vandecasteele, 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 transfer of technetium (given to the mother as pertechnetate) to the foetuses and to determine the accumulation sites in the embryo. For this purpose rats were planned to be used and possibly sheep. The long term component of Tc retention in rat was also investigated. Moreover it was planned to study the toxic effects and the possible cancerogenicity of chronic dosing of Tc to rats.

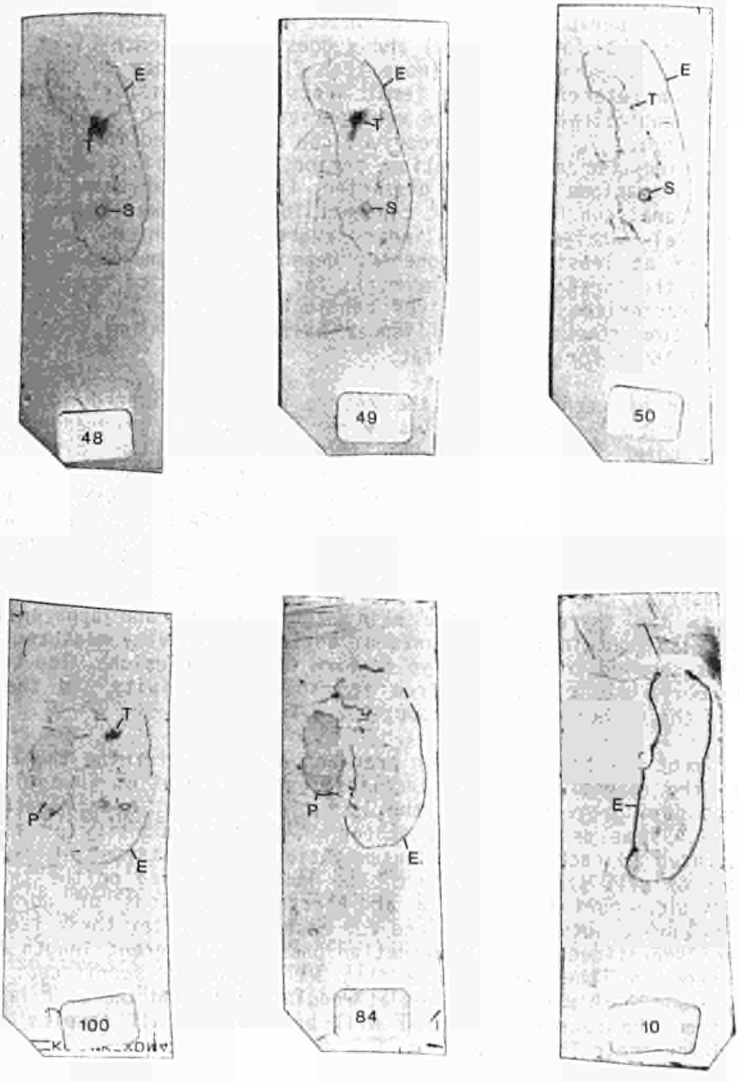
III. Progress achieved:

Based on the excretion pattern, results previously obtained and already reported regarding the retention of technetium in rats over a 7 days decontamination period allowed to estimate a first metabolic compartment of short half-life (about 0.5 d) and suggest the presence of at least a second one of longer half-life (more than 2 d). In order to characterize the long-term retention, adult female rats of the Wistar inbred R/Cnb strain were contaminated over a 5 week period with $^{99}TcO_4^-$ added to their food (6.3 MBq Tc/g). They then received non contaminated food for up to 4 months. During the decontamination period, groups of 4 animals were sacrificed at various times and dissected. Forteen organs or tissues were sampled and analysed for their Tc content. The results were submitted to a compartimental analysis (non-linear regression) which reveals the existence of at least two components. Depending on the organ or tissue considered, the first component accounts for 70 to 90 % of the body burden and is characterized by half-lives ranging from 6 to 18 h. The second biological compartment has much longer half-lives ranging from 8 d for liver up to 150 d for brain and fat.

The transfer of technetium to the embryo was investigated in the same strain of rat. Pregnant females were fed during pregnancy with a commercial diet to which Tc-95m (obtained from the cyclotron of Louvain-la- Neuve) as pertechnetate was added. Foetuses were taken out on day 21 of pregnancy and deep frozen. Some of them were used to prepare saggital sections through the whole embryo at different levels (care was taken to avoid thawing and migration of the activity). The sections (20 µm thick) were exposed in a deep freezer on a Kodak medical X-ray film. It can be observed (see photo) that most of the technetium in the foetus is located in the thyroid but the skin, the placenta and apparently the stomach, also contain large amounts of activity. Activity measurements of the organs sampled from the embryo confirm these conclusions. The transfer to the embryo was calculated from its specific activity and the daily intake of the mother and is of about 0.2 d/kg.

An experiment has been started on pregnant ewes to determine the transfer factors, the retention and the localisation of technetium in adult sheep and lambs during pregnancy and the lactation periods. A daily amount of 740 kBq Tc-99 as pertechnetate, spread on a small quantity of food, is administrated to each ewe. The contamination period started at day 50 of pregnancy or will start at birth of the lambs depending on the treatment (contamination during pregnancy and lactation periods or during the lactation period only). It will end for each mother after their lambs will have been sacrificed. A decontamination phase of different length of time is foreseen for the ewes before they will also be sacrificed. Urine, faeces, blood and wool are sampled at regular times and the most important organs from the sacrificed animal will be sampled. All samples will be analysed for their Tc content.

The study regarding the toxic effects and possible cancerogenicity planned for this year has been delayed due to a lack of man power and will be started in 1988.



Localisation of Tc-95m in foetuses
T = thyroid, P = placenta, S = stomach, E = skin
(48, 49 and 50 are successive sections from the same foetus).

IV. Objectives for the next reporting period:

The studies on the toxic effects and possible cancerogenicity of chronic administration of Tc to rats will be initiated. The transfer of technetium in utero and during lactation will be studied in sheep.

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

- Lab. de Physiologie Végétale, Univ. Catholique de Louvain, Place Croix du Sud 4, 1348 Louvain-La-Neuve
- Lab. voor Colloïdale Scheikunde, Fak. voor Landbouwwetenschappen, KUL, Cardinaal Mercierlaan 92, 3030 Heverlee
- Lab. de Biochimie, Univ. de Nantes, Chemin de la Houssinière 2, F-44072 Nantes Cedex, France

VI. Publications:

Gerber G.B., Van Hees M., Garten C.T. jr., Vandecasteele C.M., Vankerkom J., Van Bruwaene R., Kirchmann R., Colard J. and Cogneau M.
"Technetium absorption and turnover in monogastric and polygastric animals", submitted to Health Physics.

Hegela M., Vankerkom J., Gerber G.B. and Kirchmann R.
"Toxicity of technetium-99 : can it represent a risk to man", submitted to Health Physics.

Zeevaert Th., Vandecasteele C.M. and Kirchmann R.
"Assessment of the dose to man from liquid releases of 99-Tc", submitted to Health Physics.

Dehut J.P., Vanhove C., Vandecasteele C.M. and Myttenaere C.
"Technetium cycle in the environment : redistribution of bioincorporated Tc in Azolla sp. within the different compartments of a terrestrial ecosystem", Seminar on the "Cycling of long-lived radionuclides in the biosphere : observations and models", Madrid, September 15-19, 1986, in press.

Vandecasteele C.M., Capot F., Dehut J.P., Mousny J.M. and C. Myttenaere
"Technetium fate in irrigated rice fields", Seminar on the "Cycling of long-lived radionuclides in the biosphere : observations and models", Madrid, September 15-19, 1986, in press.

Kirchmann R., Fagniart E.
"Transfer factor values observed in experimental field conditions and from Chernobyl fall-out", Workshop Soil-Plant Transfer Factors of Radionuclides organized by the UIR, Egham, Surrey, U.K., April 13-16, 1987, in press.
Vandecasteele C.M.
"Influence du technetium sur la nitrogénase d' Azotobacter". Thèse de doctorat, Université Catholique de Louvain, Louvain-la-Neuve, 1987, 178 pp.

Title of the project no.:

2
Comparative study of the radioecology of the continental water of the Meuse and Rhône basins

Head(s) of project:

C. Vandecasteele

Scientific staff:

R. Kirchmann, J.R. Maisin, E. Fagniart, E. Bonnijns, J.P. Descy, M. Meurice- Bourdon, J.M. Théate, J. Smitz, E. Everbecq, L. Sombré, J.C. Micha, A. Gillet, H. De Clercq-Versele, J. L. Avaux, G. Beuken

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 objectives of this research program is to gain a better understanding regarding the behaviour of radionuclides released into freshwater ecosystems and to gather a more accurate knowledge on their transfer to man through the food chain. The ultime objective is the building of a general transfer model in fresh water 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 Rhône basin (French contribution) ; the data and models obtained for both rivers will be compared.

II. Objectives for the reporting period:

- Obtainment of SITE SPECIFIC DATA for the Meuse ecosystem : determination of the diet composition of the perch (Perca fluviatilis), a secondary consumer and collection of various aquatic plant and animal species for the determination by gamma spectrometry of their radiocontamination levels.

- LABORATORY STUDIES on the transfer of ^{60}Co and ^{134}Cs from water to a diatom representative of the Meuse phytoplankton and on the contamination of algal cultures grown on effluents from a PWR. Study of the transfer of radiocaesium from water or through the foodchain to the barbel (Barbus barbus).

- MODELLING of the contamination/decontamination process for ^{60}Co in aquatic ecosystems ; carrying on of the development of the general radioecological model for freshwater ecosystems and of the feeding of the data base.

III. Progress achieved:

1. Field studies

Several sampling campaigns have been organized to collect aquatic plants and animals from the Meuse on the Belgian territory. Moreover a survey of the radiocontamination of the bottom sediments has been carried out in August 1987 in the Meuse river between Givet at the French boarder and the first sluice-gate (Grande Malade) downstream the junction between the Meuse and Sambre rivers. This survey was performed in the framework of the CEE coordinated programme in collaboration with the CEA Cadarache and the CEA Toulon, using an immersed NaI detector trailed by the CEA scientific boat "Ecologix". At various stations core and bulk sediments have been sampled for radiocontamination analyses. The measurements are under course and will be provided in the next progress report.

The composition of the diet of the perch (Perca fluviatilis) has been completed. This fish is a bottom feeder and feeds principally on crustaceans (Asellus, Gammarus, ...) and on various insect grubs. Only individuals bigger than 18 cm feed on fish, mostly on bleaks (Alburnus alburnus).

2. Laboratory studies

The studies on the contamination of a diatom representative of the Meuse phytoplankton (Cyclotella meneghiniana) have been carried on. The results obtained last year regarding the contamination of this alga by Co-60 have been confirmed : the uptake of Co by the algae is very rapid (more than one half of the maximum activity taken up by the cells are fixed within a few minutes) and an equilibrium is reached after about 10 hours ; at this time the transfer factors calculated range between 5000 and 13000.

The same kind of experiments performed with Cs-134 indicates that the incorporation of this element into the algal biomass is also very rapid. However, all data are not yet available so that no firm conclusions can be drawn actually.

The studies regarding the transfer of radiocaesium in an experimental fresh water food chain (water-algae-fresh water mussels (Dreissena polymorpha) fish (Barbus barbus)) have been carried on, focussing the attention on the last link of the chain. The results obtained and their mathematical treatment show that for long-term period the transfer via the feed (contaminated mussels) is more important than the water pathway and contributes to 50 to 90% of the activity accumulated by the fish ; it was also calculated that, in the case of an accidental release, the food pathway would already contribute one year after the release, for more than 70% of the fish contamination level.

When considering accumulation of radiocaesium by the fish from both water and food under routine release conditions, the transfer factors calculated by the model, related to the water contamination level reach after a five year equilibration period values of about 400 to 600 on a dry weight basis 80 to 120 on a fresh weight basis. These values are of the same order of magnitude as the lower limit of range of transfer factors measured for fish in the environment. That means that the transfer parameters (obtained from controlled experiments) and/or the mathematical model used underestimate the accumulation in the fish.

In order to perform experimental studies under conditions closer to the real world, an experimental river model is under construction. This system simulating the flow and the structure of a real river (presence of bottom sediments and suspended matter) will allow to check laboratory data previously obtained and to take into consideration the rôle of the solid phase.

3. Modelling

Mathematical models have been built that describe the phenomena involved in the contamination/decontamination processes for Co-60 in freshwater ecosystems. These models were run with experimental data obtained on algae at the Laboratoire des Eaux Continentales of the CEA-Cadarache and data from the Laboratory of Radioecology of the U.Lg. (Liège). Contrary to what was observed for Cs-134 of which accumulation could be interpreted solely by surface phenomena, the accumulation of Co-60 is dependent of the biological activity of the cultures.

The development of the general radioecological model has been carried on. It is facing a gap of information regarding the recycling of the activity accumulated during the life of organisms, after these have died and concerning the rôle of the bacterial flora of the sediments in the detrification processes leading potentially to the recycling of the radionuclides associated with the solid (mineral and organic) phase. In order to be able to estimate the importance of these generally neglected phenomena and to fulfil the parameter requirements of conservation equations to close the radionuclide cycle, several bacterial strains associated with diatom populations or living in the water or in the sediments will be isolated and purified ; the rôle of these microorganisms in the accumulation of radioactivity from water and in the remobilisation processes will be initiated in 1988.

IV. Objectives for the next reporting period:

- Various fish species will be collected twice a year from three sampling stations on the Meuse river and analysed for their radioactivity content.
- Carrying on of the experimental studies on the transfer to diatoms of Co and Cs from real effluents of a PWR. Starting of the studies on the rôle of bacteria : isolation, purification, identification and preliminary experiments. Improving the experimental river model and starting of the experimental work regarding the distribution of activity between the solid and liquid phases before introducing biological compartments. Study of the transfer between fish (prey) and a canivorous fish.
- Modelling of the experimental results and development of the general radioecological model focussing on the transfer of Co-60 up to the fish compartment.

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
- Lab. de Physiologie Végétale, Univ. Catholique de Louvain, Louvain-la-Neuve
- Lab. de Radioécologie, Univ. de Liège, Sart Tilman (Liége)
- Unité de Modélisation Mathématique des Eaux Intérieures, Univ. de Liège, Sart Tilman (Liége)
- I.H.E., Bruxelles
- The Centrale Nucléaire de Tihange (SEMO) actively cooperates to the present project
- Lab. d'Etude de la Pollution des Eaux, CEN/CEA, Cadarache

VI. Publications:

Detollenaere A., Micha J.C. (1986)
Impact des rejets thermiques de la centrale nucléaire de Tihange sur les poissons de la Meuse. Tib. Cebedeau, 516, 39, 9-26.

Meurisse-Genin M., Reydams-Detollenaere A., Stroot Ph. et Micha J.C. (1987)
Les macroinvertébrés benthiques de la Meuse belge : bilan de cinq années de recherches (1980 à 1984). Arch. Hydrobiol., 109, 1, 67-88.

Galvez M. et Micha J.C. (1987)
Introduction, extension et répartition du sandre en Belgique. Trib. Cebedeau, 521, 33-42.

Gillet A. et Micha J.C.
Biologie et radiocontamination de 3 espèces animales (Dreissena polymorpha P., Rutilis rutilus (L.) et Perca fluviatilis (L.) représentatives de différents maillons trophiques de l'écosystème Mese. Ann. Assoc. Belge Radioprotection, Vol. 12 (2-3), in press.

Descy J.P.
Etudes écologiques de la Meuse en relation avec les rejets des centrales nucléaires. Ann. Assoc. Belge Radioprotection, Vol 12 (2-3), in press.

Debauche A. et Descy J.P.
Radiological monitoring in the Belgian part of the river Meuse (1984) : results in the aquatic mosses. UIR, 8th annual meeting, Brussels, 1985.

Sombre L. (1987)
Contribution à l'étude du transfert du radiocésium (^{134}Cs et ^{137}Cs) dans une chaîne alimentaire d'eau douce simplifiée : eau-algue verte (Scenedesmus obliquus) - mollusque filtreur (Dreissena polymorpha). Thèse de doctorat, Faculté des Sciences Saint Charles, Université de Provence (Aix-Marseille I), 146 pp.

CEN/SCK (1987)
Etude comparée de la radioécologie des eaux continentales des bassins mosan et rhodanien, contribution des laboratoires belges. Rapport technique d'avancement 1986, 191 pp.

Sombré L., S. Carrao et C. Myttenaere
Transfert du 134-Cs dans une chaîne alimentaire d'eau douce simplifiée : eau-algue verte (Scenedesmus obliquus) - mollusque filtreur (Dreissena polymorpha).
Ann. Assoc. Belge Radioprotection, Vol. 12 (2-3), in press.

Sombré L., S. Carraro et C. Myttenaere
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, Vol. 12 (2-3), in press.

Smitz J.S., E. Everbecq et B. Comeliau
Modélisation du transfert du 137-Cs de l'eau vers le phytoplancton et simulation des expérimentations de laboratoire.
Ann. Assoc. Belge Radioprotection, Vol. 12 (2-3), 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 (3-H and 14-C transfer in mammals)

Scientific staff:

G. Gerber, M. Van Hees, M. Mergeay
H. De Clercq-Versele
M. Meurice-Bourdon, J. Vankerkom, R. Kirchmann

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 of the formation and release of OBT by degradation of resins used for water purification in nuclear power plants and of 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 microbiological mechanisms of tritium (especially HT) uptake by soils and on elementary tritium contamination of plants,
- the transfer of tritium under various chemical forms and 14-C to mammals (mono- and polygastric) is studied in order to predict the behaviour of OBT in man.

II. Objectives for the reporting period:

- Study of the incorporation of ^{3}H from HTO into the organic compounds of a red strain of yeast, isolated from the primary cooling system of the BR2 (Belgian Reactor 2), by column chromatography and gel electrophoresis. Investigation on the release of tritiated molecules from the yeast cells, and from the purification resins, submitted to irradiation.

- Studies under controlled conditions of the mechanisms responsible for the H-3 contamination of plants and bacteria exposed to HT. Participation to the French HT release experiment. Production of C-14 contaminated plant material for food chain transfer studies.

- Studies of the transfer of various forms of OBT (meat, milk powder, milk constituants) to rat.

III. Progress achieved:

1. Source-term

A red yeast isolated from the primary loop of the BR2 was used to investigate the incorporation of tritium supplied as HTO into organic molecules.

The yeast was grown in an artifical medium (Medium 869) in presence of different HTO contamination levels, from 0 up to 37 kBq/ml. After an incubation period of one week the cells were harvested by centrifugation, repeatedly washed and processed for tritium analysis by microcombustion. Under these artificial growth conditions the incorporation of tritium into the total yeast organic matter increased linearly as a function of the specific activity of the liquid medium. Moreover, it was observed that about 13% of the newly synthesized organic matter was excreted in the culture medium. The excreted tritiated material is constituted by high molecular weight organic compounds, insoluble in 10% cold TCA as well as by small molecules. Using a suitable buffer, it was possible to extract from broken cells about 85% of the tritium labelled compounds, a relatively small fraction (15%) remains associated with the insoluble pellet. Column chromatography analyses of the tritiated extracts revealed that most of the radioactivity (80 to 90%) was represented by organic molecules having a molecular weight lower than 10,000 daltons.

These results show that the red yeast, originally found in the primary cooling system of a PWR, is able to produce tritiated organic compounds when supplied with HTO and to excrete part of the labelled material into the external medium. It is of course expected that a further release will occur by lysis of the cells when the yeast die. It is, thus, important to determine the life span of red yeast cells, by using an experimental device simulating the flow of water in the primary loop through the decontamination resins. The chemical nature of the organic compounds produced by the yeast cells is also worth studying.

2. Dynamic models in soil plant systems

Plants exposed to tritiated hydrogen (HT) released from nuclear installations or under experimental conditions were demonstrated to contain tritium in their biomass both as tritiated tissue water (HTO) and organically bound tritium (OBT). The question of whether the plants only incorporate HTO produced by the oxidation of HT in the atmosphere or, more intensively, in soils or whether they are able to oxidize and metabolize HT by themselves was raised. To answer this question plants were grown on nutrient solutions (in absence of any soil system) and exposed in a glove box during three hours to tritiated hydrogen (0.5 MBq HT/l atmosphere). Care was taken to avoid the presence of HTO as a contaminant in the HT gas injected by washing the tritiated gas with water prior to injection into the experimental system containing the plants. The concentration and speciation of tritium in the confined atmosphere during exposure was continuously monitored by sampling the atmosphere of the glove box and discriminating between HT and HTO. HTO present in the air was first trapped by bubbling in ethyleneglycol before HT remaining in the gas phase was oxidized at 600°C with Pd catalyst before being trapped as HTO by bubbling in ethylene glycol. The tritium incorporated in plants was analysed by distillation with toluol for HTO and by microcombustion for OBT (organically bound tritium). Immediately after exposure it was observed that most of the activity taken up by the plant was present as HTO (about 3.5 and 0.9 kBq ml tissue water in the aerial parts and roots respectively) ; less than 1% was present as OBT. The results also showed that the HTO content in the atmosphere of the glove box remained very low and could not explain the amount of ^{3}H incorporated by plants. It was also

evident from the data that a transfer of HTO from the nutrient solution to the plant through the root system could also be excluded ; on the contrary some excretion of HTO from the roots to the nutrient solution was observed in plots of which the root compartment was isolated from the contaminated atmosphere. We must then conclude that plants are able to oxidize HT at the level of their aerial parts and to transfer part of the HTO formed to the root systems, but until now the mechanisms involved are not elucidated.
Several plant species of economical interest (potatoes, wheat, grass, clover, cabbage, ...) were grown on soil in order to be exposed in France (Bruyère-le-Châtel) to a HT release under real environmental conditions. For various reasons (climatic and others) the French release experiment could not happen in 1987 and was delayed.

3. ^{3}H and ^{14}C transfer in mammals

In the framework of a collaboration with the laboratory for Animal Physiology of the Landbouwhogeschool at Wageningen (Prof. J. Van den Hoek), C-14 contaminated forage was produced using the facilities of the experimental farm of the CEN/SCK. A 50 m^2 surface of grass-land was exposed under a tightly closed plastic greenhouse (volume of about 100 m^3) to 185 MBq of $^{14}CO_2$ (puff release). During the 24 hours following the injection of the radioactive gas, stable CO_2 was added to the close system from a cylinder to restore CO_2 levels suitable for plant photosynthesis as well as to increase the uptake of $^{14}CO_2$ remaining in the atmosphere. After this 24 hours confinement period, the plastic greenhouse was removed and the grass was harvested three days later. The contaminated forage (65.9 kg fresh weight) was dried and stored to be used in experiments studying the metabolism of C-14 in lactating ruminants.
On the other hand, the various organs collected at sacrifice from a cow and from a young bull contaminated last year with tritium as HTO in order to produce tritiated animal products (milk and meat) were analysed for their tritium contamination level as OBT (organically bound tritium) by microcombustion. It was observed that the OBT concentration measured in the organs from the cow (which received a total amount of 74 GBq HTO, equally distributed over a 30 days period and was sacrificed at the end of dosing) varied between 0.2 up to 12 kBq/g O.M. (organic matter) depending on the organ considered, the less contaminated ones appeared to be the skin, the fat, the bones and the bone marrow, all tissues that are characterized by a low metabolism and a high content in constituants with low turn-over rates. The OBT content in samples obtained from the cow was highest in the bile (11.7 kBq/g O.M.), followed by the duodenum (6.7 kBq/g O.M.), the jejunum (5.2 kBq/g O.M.) and the liver (6.0 kBq/g O.M.) ; most of the organs however ranged from 3 to 6 kBq/g O.M. The OBT concentration measured for the organs of the bull (which received in a unique administration the same total amount of HTO as the cow and was slaughtered three days after dosing) were in general 2 to 4 times higher than those measured for the cow and ranged from 0.4 to 17.2 kBq/g O.M. The less contaminated fractions were the fat and the bone marrow, the highest ones were the liver and the bile ; OBT activities in most fractions ranged from 8 to 12 kBq/g O.M. As it could be expected the activity measured in the fat compared to that in the muscles was proportionally much lower in the bull shortly after an acute contamination than in the cow for which the exposure time was longer. It can thus be expected that the distribution of OBT between the main constituants (glucides, proteins, and lipids) will be different depending on the contamination scenario. It will be of interest to investigate the incidence of this distribution in the meat on its further transfer in the food chain. This part of the work will

be initiated in 1988, since in 1987, a lack of man power did not allow to perform, as foreseen in the time schedule, the studies on the transfer to rat of the various forms of ORT obtained from the experiment reported here above.

IV. Objectives for the next reporting period:

- Determination of metabolic activity and life span of yeast cells in a simulated reactor primary circuit. Study of the chemical nature of the tritiated molecules produced by yeast cells. Study of the microbial population living in the primary and secondary loops of a PWR, including purification resins.
- Studies under controlled conditions of the mechanisms involved in the HT incorporation by plants.
- Studies on the transfer through the food chain of various forms of OBT (meat, milk powder, milk constituants). Preliminary experiments on rats.

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

Dieren Physiologie Lab., Landbouwhoeschool, Wageningen
SERE-IPSN-DPS, CEA Fontenay-aux-Roses
Institut d'Hygiène et d'Epidémiologie, Bruxelles
Unité de Radioécologie, Université de Liège, Sart Tilman

VI. Publications:

Fagniart E., Van Hees M. and Kirchmann R.
"Study on the persistance of C-14 in an agricultural ecosystem. ESNA XVIIth Annual Meeting, Hannover, September 14-19, 1986, in press.
Kirchmann R.
"Study on the persistance of C-14 in an agricultural ecosystem". Progress Report IAEA coordinated Research Programme on "Carbon-14" from Nuclear Facilities", third meeting, San Carlos de Bariloche, November 24-28, 1986, in press.
Vandecasteele C.M.
"Transfert dans les écosystèmes terrestres : écosystèmes agricoles". Communication Journée Interuniversitaire d'Information : "Le nucléaire et l'environnement", Louvain-La-Neuve, 25 avril 1987 (unpublished)

Working Group Meeting of the coordinated tritium programme of the CEC. CEN/SCK, Mol, 21-22 May 1987, report in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 of HTO/HT/OBT
(Experimental studies and modeling)

A: HTO-transfer in the atmosphere-soil system;
B: HT-deposition and conversion in soils.

Head(s) of project:

Dr. C. Bunnenberg

Scientific staff:

Dr. C. Bunnenberg

Dipl. Phys. J. Feinhals

Dipl. Phys. B. Wiener

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 study exchange processes of HTO and H_2O between atmosphere and soil and the effects of soil porosity, temperature and exposure time on the HTO deposition on soils.

Mathematical description of the obtained relationships.

B. Participation in a HT field experiment in Canada, evaluation of the measurements and comparison with the results obtained in the French experiment.

Completion and test-runs of the laboratory set-up to study HTO reemission under controlled conditions.

III. Progress achieved:

A. HTO-transfer in the atmosphere-soil system

1. Methodology

Water vapor exchange between atmosphere and soil leads to a deposition of HTO on soils, which may be more effective than washout processes. It was found that this is due to the fact that HTO and H_2O molecules follow their individual vapor pressure gradients. Consequently, the tritium concentration [HTO] of the deposited moisture gain may be higher than that of the atmospheric humidity, expressed by the specific activity ratio k:

$$k = [HTO]_{gain} / [HTO]_{air} \qquad (1)$$

As this ratio represents an important value for predictions of soil contaminations on the basis of condensation rates, laboratory experiments are performed with soil columns and model atmospheres, to investigate HTO and H_2O depositions on soils under different meteorological and soil physical conditions and the effect on k.

2. Results

While preceding experiments have shown that the specific activity ratio may rise well above one with increasing soil moisture θ with a pronounced dependence upon the soil type, recent investigations confirmed that k is independent from soil porosity, soil temperature and duration of the deposition process. On the basis of these findings it is possible to describe the deposition processes of both types of molecules in a convenient manner with help of a fallout model and Fick's first law. The flux $\vec{J}$ of HTO or H_2O between atmosphere and soil is:

$$\vec{J} = \vec{v} \cdot C = - D_{\theta v} \cdot \text{grad } C \qquad (2)$$

with the vapor diffusion coefficient $D_{\theta v}$ and the concentration C of HTO or H_2O in air near the soil surface. Equ. (2) also defines deposition velocities of the respective types of molecules. Neglecting the small difference of $D_{\theta v}$ for HTO and H_2O the relationship leads to another expression for the specific activity ratio:

$$\vec{v}_{HTO} = k \cdot \vec{v}_{H_2O} \quad , \qquad (3)$$

which establishes a connection between the static observation (Equ. 1) and the process of deposition as a next step towards a dynamic model.

3. Discussion

The given equations demonstrate the central importance of the specific activity ratio k. According to the observations k may be greater or smaller than one. It may even be negative, indicating transports of HTO and H_2O in opposite directions. Therefore, the previously used notation "accumulation factor" has been replaced by "specific activity ratio", to cover all possible cases. It is anticipated to derive relationships, to deduce deposition velocities from macroscopically observable parameters on the basis of Equ. 2.

B. HT deposition and conversion in soils

1. Methodology

It was found from laboratory experiments that elemental tritium is oxidized to tritiated water in contact with soils because of microbiological actions. In 1986 (France) and 1987 (Canada) two field experiments have taken place to study the fate of tritium after releases of HT into the atmosphere under natural conditions. The experiments differed in season, weather conditions, release height and duration. Main objectives of NIR participation were: dispersion of the HT plume, HT deposition and conversion into HTO in contact with soil, HTO diffusion in soil and reemission, dispersion of the HTO plume from the area source and redeposition of HTO on soils. All processes contribute to the local and time variations of the HT and HTO concentrations in air and soil, which lead to short and long-term dose consequences.

2. Results

The Canadian experiment has confirmed the experiences from the French release that the HT dispersion can be described by the Gaussian plume model, when the dispersion parameters are properly chosen. On the basis of measurements of the time-integrated HT concentrations in air and corresponding HTO contents in soil the range of deposition velocities v_{HT} across the experimental site was evaluated, showing variations between $0.8 \cdot 10^{-4}$ and $7.1 \cdot 10^{-4}$ m $\cdot$ s^{-1}. On freshly disturbed soil plots considerably lower deposition velocities were found, leading to a lower contamination of those areas. This effect must be attributed to the disturbance of the microbiological profile in soils with the consequence that because of the soil manipulations less vital HT-oxidizing bacteria are transferred to the soil

surface, and HT is offered before they can restore the natural vitality profile.

During passage of the HT plume a HTO contamination of the top soil layers occurred, giving rise to HTO diffusion in soil and reemission from the surface according to the concentration gradients and the meteorological conditions. With the assumption of an exponential decrease of the soil HTO the average reemission rate was $r_{HTO} = 1.2\ \% \cdot h^{-1}$ during the first 24 h after release. Afterwards a heavy rainfall washed down the soil HTO into deeper layers, diluting concentrations in the top soil. As a consequence reemission rates decreased significantly. The resulting concentrations of air HTO reflected the changes of the reemission from the area source. It was also observed that there is a general tendency of higher air HTO concentrations during the day than at night. This is due to the pronounced day-night cycle of evaporation and condensation periods.

Redeposition of air HTO on areas of lower primary contamination was determined yielding a mean HTO deposition velocity $v_{HTO} = 4 \cdot 10^{-3}\ m \cdot s^{-1}$.

3. Discussion

The Canadian field experiment added valuable new information on tritium transfer mechanisms concerning the overall effect on concentrations in the air and soil compartments under natural conditions as well as the individual processes involved. Special relationships to environmental parameters, however, can only be deduced from laboratory experiments under controlled conditions. Therefore, a soil column/wind tunnel set-up has been completed and tested to investigate the effect of single parameters on the HTO reemission from soils.

IV. Objectives for the next 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.

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

L.A. König, H. Schüttelkopf, S. Diabaté, KFK, D-7500 Karlsruhe, FRG
G. Ogram, F. Spencer, CFFTP, Ontario Hydro, Toronto, Canada
R. Brown, AECL, Chalk River, Canada
O. Edlund, Energiteknik, Studsvik, Sweden
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Y. Belot, CEA/IPSN, Fontenay-aux Roses, France
J. van den Hoek, Agricultural University, Wageningen, Netherland
R. Kirchmann, CEN/SCK, Mol, Belgium

VI. Publications:

(1) Bunnenberg, C.; Feinhals, J.; Wiener, B.
Differences in the behaviour of HTO and H_2O in soil after condensation from the atmosphere and conversion of HT to HTO and OBT in soil relative to moisture content and pore volume.
Rad. Prot. Dosimetry, Vol. 16, No. 1-2 (1986), 83-87.

(2) Bunnenberg, C.; Wiener, B.; Kühn, W.
Feldmessungen zur HTO-Reemission vom Boden (Kurzbericht).
84. Sitzung des Radioökologieausschusses der SSK, Bonn, 1987.

(3) Bunnenberg, C.; Feinhals, J.; Wiener, B.
Dynamic environmental cycling of HT/HTO/OBT,
Recent results of the NIR-contribution to the coordinated program.
Tritium Coordination Meeting, Brussels, May 21-22, 1987.

(4) Wiener, B.; Täschner, M.; Bunnenberg, C.
NIR results on the Canadian HT experiment.
OHRD Draft Report, Dec. 1987.

(5) Bunnenberg, C.; Feinhals, J.
HTO-Austausch im System Atmosphäre-Boden.
NIR-Jahresbericht 1987 (in press).

(6) Bunnenberg, C.; Wiener, B.
Umsetzung von HT zu HTO und OBT im Boden.
NIR-Jahresbericht 1987 (in press).

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

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). Investigations 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.

B. Inventory of I-129 and I-127 in human thyroid glands collected during the reporting period.

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 soils were continued. For comparison two different soil types had been labeled: a sandy soil (undisturbed soil monolith) and an allochthone silty loam on a river bank (pasture). Soil samples were taken in 5 and 10-cm steps down to 100 cm and analysed for I-129 contents.

2. Results

Iodine translocation is very slow in the monolith. Even 52 months after I-application 88 % of the activity can still be found in the top 10-cm soil layer. In the case of the allochthone soil, 82 % of the activity are found down to 20 cm, 35 months after labeling, indicating a considerably faster transport of iodine in this specific soil. Parallel investigations of the K_d-values of those soils confirm a slight tendency of lower values for the silty loam. The differences, however, are too small for a satisfactory explanation of the observed translocation. They more likely suggest a mass transport of non-biochemical nature.

3. Discussion

The deeper penetration of iodine in the allochthone silty loam gives rise to more thorough investigations on the nature of the observed transport, in order to estimate the frequency of possible occurrence.
Because of a longer maintenance shut-down of the reactor needed for the analysis of low-activity samples, the corresponding plant samples could not be evaluated for the determination of transfer coefficients. The same refers to samples from the "in vivo" experiment on the transfer of I-129 to milk and beef. Those measurements will be brought-up in the next reporting period.

B. Inventory of I-129 and I-127 in human thyroid glands

1. Methodology

Seven thyroid glands from different parts of Lower Saxony, FRG, were analysed for I-129 and I-127 in 1987. As with the previous investigations the organs were taken during autopsy of selected individuals. Only those organs were analysed, that had not been affected by any desease or therapy of the deceased.

2. Results

The isotopic ratio I-129/I-127 of the investigated glands varies between $2.3 \cdot 10^{-8}$ and $9.5 \cdot 10^{-8}$ with a mean value of $5.6 \cdot 10^{-8}$. The values are essentially similar to those obtained during the past five years.

3. Discussion

The long-term stability of the I-129/I-127 ratio demonstrates a rather constant level of I-129 in a region without direct radioactive impacts inspite of the Chernobyl accident.

IV. Objectives for the next reporting period:

Investigations on the transfer coefficient soil to plant of I-129 with the '87 and '88 samples from the two soil types and on the translocation.

Evaluation of grass and milk samples collected in a long-term "in vivo" experiment and determination of the transfer along the food chain.

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

D. Smidt, F.-W. Huth, A. Pfau, Institut für Tierzuchtverhalten der FAL, Mariensee, FRG

A. Georgii, Pathologisches Institut der Medizinischen Hochschule, Hannover, FRG

VI. Publications:

(1) Handl, J.; Pfau, A.
Feed-milk transfer of fission products following the Chernobyl accident.
Atomkernenergie-Kerntechnik Vol. 49, No. 3, 1987.

(2) Kühn, W.; Handl, J.
Contamination of milk and meat and radiation exposure as a result of winter feeding.
CEC-Workshop on the assessment of radiological and economical consequences.
Brussels, February 3-5, 1987.

(3) Handl, J.
Transfer of radionuclides from plant to animal resulting from the Chernobyl accident.
Conference of the Working Group Plant-Animal, UIR, Grange-over-Sands Cumbria, UK, October 19-23, 1987.

(4) Handl, J.; Pfau, A.
Untersuchungen zum Transfer von Radiocaesium und K-40 vom Futter zu Rind, Milch und Kalb nach dem Tschernobyl-Unfall.
7. Fachgespräch zur Überwachung der Umweltradioaktivität, München, 16.-17. November 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Contract no.: B16-B-194-UK

Associated Nuclear Services
Eastleigh House
60 East Street
GB- Epsom, Surrey KT17 1HA

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

Dr. A. Martin
Environmental Safety Department
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. 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 the chemical extraction of C-14 from labelled plant and soil materials and to test and operate laboratory facilities for controlled investigations on transfers of C-14 from soil to vegetation via root uptake.

(ii) To use these facilities and techniques to follow the transfer of C-14 from soil solution to plant roots in laboratory conditions and in soil-plant systems maintained in controlled glasshouse systems.

(iii) To derive rate-coefficients for C-14 transfer in soil-plant systems which can be used to further develop and test dynamic models forming part of the SPADE suite of codes.

III Progress achieved:

1. Methodology

Emphasis has continued to be placed on the design, construction and testing of equipment which can be used for laboratory and greenhouse investigations on transfer of C-14 from soil to plant via root uptake.

The solution-culture apparatus described in the previous progress report has been modified further to ensure complete containment of administered C-14. A further experiment has been undertaken using this modified experimental arrangement. This involved the exposure of *Lolium perenne* to C-14 administered to culture solution as bicarbonate as a single input at time zero. Sampling was undertaken for 48 hours post-administration and, during this period, top and bottom chambers were alternately ventilated with samples obtained of nutrient solutions, and bottom and top-chamber ventilation streams. C-14 was also determined in plant root and shoot components at the termination of the experiment. In addition to the three replicate exposure systems used to follow C-14, two further control systems were introduced. One was treated identically except that no plants were included in the system, the other was treated identically except that no C-14 was introduced directly.

The soil-exposure system described in the previous progress report was subjected to slight modification and used for a further experiment with *Lolium perenne*. This involved the administration of C-14 to six exposure systems at time zero with subsequent frequent analysis of C-14 in the ventilation stream passing over the soil surface. Three systems were harvested destructively at 29 hours post-administration and the remaining three at 75 d post-administration.

2. Results

Previous nutrient solution studies indicated that ∿0.32% of a spike of administered C-14 was taken up and retained by *L. perenne* shoots over a 40 h period. The recent studies show a range in values of 0.29 to 1.0% of administered C-14 in *L. perenne* following 48 h exposure with no C-14 detectable in the control system. Corresponding concentration ratios over initial solution concentrations were 20 to 45. 1.4 to 9% of administered C-14 was recovered in the ventilation stream passing through the top chamber and representing respiratory losses from shoots. The appearance of some C-14 in the ventilation stream from the control system containing no plants indicates that further testing of the exposure system is necessary before fully conclusive results can be provided.

C-14 administered to the soil exposure system is lost rapidly. In the most recent experiment; losses via the soil ventilation stream reached 35% of the administered activity by 29 hours post-administration. At this time plants on the contaminated half of the exposure system accounted for ∿0.02% of administered C-14. C-14 was detected in plants on the uncontaminated half of the system and, at very low levels, in the underlying soil. Corresponding concentra-

tion ratios for plant shoots on the contaminated half of the system were 6.0 to 6.8. C-14 in plants harvested at 75 d post-administration had fallen by an order of magnitude compared with values obtained at 29 hours.

Discussion

Results have been obtained and analysed for a series of two culture solution experiments using a complex exposure and containment system. The results obtained generally confirm those using simpler exposure systems and provide some indications of root uptake of C-14 by *L. perenne* from solution. Though the uptake is relatively small compared with the total administered it leads to high shoot/solution concentration ratios as a result of rapid declines in solution content with time. The results also indicate that further testing of the exposure system would be advantageous so that conclusive results on rates of transport can be derived.

Results of a series of three soil exposure experiments using *L. perenne* are broadly comparable. Though administered C-14 is, as expected, lost rapidly from the exposed soil, a small proportion is transferred rapidly to plant roots and translocated to shoots. After this initial transfer, C-14 in shoots is lost via respiration and this provides an opportunity to obtain information on translocation rates and losses to soil from roots in the uncontaminated half of the exposure system.

The results obtained from the recent series of experiments are being used to provide data sources for dynamic models describing C-14 transfers in soil-plant systems as part of a parallel series of projects undertaken by ANS for the UK Ministry of Agriculture, Fisheries and Food.

IV Objectives for the next reporting period:

Further solution-culture and soil exposure experiments will be undertaken to confirm the rates of transport of C-14 from soil to plants via root uptake. As previously, the objective of these experiments will be to determine the time-dependent characteristics of C-14 transfers in soil-plant systems and within various metabolic pools of the plant. This will supplement the extensive literature which exists on C-14 distribution and metabolism in plants following fixation from atmosphere and provide a basis for revised models for C-14 transfer in soil-plant systems.

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

The work reported here is being undertaken in collaboration with, and using facilities at:

Department of Botany,
University of Bristol,
Woodland Road,
Bristol, BS8 1UG.

(Dr. M.H. Martin)

Department of Chemistry,
University of Surrey,
Guildford,
Surrey, GU2 5XH

The work is supported by the Ministry of Agriculture, Fisheries and Food, Food Science Division.

VI Publications:

Since the main emphasis in the current period has been on the further evaluation of experimental and analytical techniques, no further publications have been prepared or issued. This situation is expected to be rectified during the next reporting period.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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

Scientific staff: Miss A. Hayes

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 computer models for the distribution of radionuclides in the Irish marine environment.

II. Objectives for the reporting period:

The main objectives for the reporting period were to establish the disappearance of the Chernobyl fallout from fucus seaweed and to continue measurement of the concentrations of caesium isotopes in seaweed and water which were attributable to the Sellafield discharges. Further work was to be carried out on intercalibration measurements with other laboratories. Work was to be carried out on using data obtained in computer models and in determining the best modelling procedures for caesium in the Irish Sea.

III. Progress achieved:

(1) Methodology

Measurement of samples of fucus vesiculosis collected at four sampling points along the East coast of Ireland continued throughout the year. Measurement of water samples was also carried out.

(2) Results

The mean values for 137-caesium activity in fucus seaweed at the North Dublin station were as follows.

1984	99 Bq/kg dry weight
1985	78 Bq/kg dry weight
1987	52 Bq/kg dry weight

The 1986 figures are not included as they showed a substantial disturbance due to the Chernobyl accident; this did not contribute significantly in 1987.

Seawater samples from the Irish Sea have shown a slight drop on the earlier figures, though a wider spread of values (from 160 to 200 mBq/litre) was recorded. However, the ratio of 137-caesium to 134-caesium in seawater was found to average 16 during the year for samples from the North Dublin sampling station. This is not in accordance with the ratio found in earlier years or with the value expected from the known ratio of the isotopes in the Sellafield discharge. Further intercalibration exercises were carried out with other Irish laboratories and with laboratories in the United Kingdom and Denmark. These indicated excellent agreement between measurements made in the different laboratories. Data from this project has been made available to the European Commission MARINA Committee and will be incorporated in the database and models developed by this Committee.

(3) Discussion

It is clear from the measurements on both seaweed and water that the amount of 137-caesium in the Irish Sea is decreasing. The ratio of the caesium isotopes is disturbed from its expected value and this can be attributed to lingering effects of the fallout from the Chernobyl accident. The total inventory of 137-caesium has not significantly changed as a result of the accident but the disproportionate effect on the 134-caesium levels is likely to persist for some time.

In general, the objectives for the period have been achieved. The use of the data in model verification is proceeding largely through the MARINA group and it seems likely that this should continue as it presents the best way of making use of existing expertise in this field.

IV. Objectives for the next reporting period:

Sampling at the selected stations during 1988 will be continued. It is proposed to concentrate the sampling on the stations in the North Irish Sea to investigate as closely as possible the variation in the caesium ratio during the year, in addition to following the absolute 137-caesium levels.

The United Kingdom M.A.F.F. laboratory at Lowestoft is organising a further intercalibration exercise and this will be participated in.

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.
De. A. Aarkrog,	Risø National Laboratory DK-4000, Roskilde, Denmark.

VI. Publications:

"Fucus Vesiculosis as an indicator for caesium isotopes in Irish coastal water".
I.R. McAulay and D. Pollard.
Paper presented to the International Seminar on Radioactivity and Oceanography at Cherbourg, 1987 (in press).

Title of the project no : 2.

Radioactivity in Foodstuffs Produced in the Republic of Ireland.

Head(s) of project: Dr. I.R. McAulay

Scientific staff: Mr. D. Moran
Miss A. Hayes

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 radio-isotopes 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:

Measurements of radioactive content of a range of food products. Detailed soil measurement at ten sites in the Republic of Ireland. Identification of areas of particular interest and more detailed study of these. Assessment of population dose due to the radioactive content of foodstuffs. Intercalibration of measuring equipment with other laboratories.

III. Progress achieved:

1. Methodology.

Measurements have been made of soil samples at 5 cm intervals of depth at ten sites distributed around Ireland. Samples of different types of crop have been measured at each of the ten sites, together with samples of tilled soil in which the crops have been grown. Some additional sites have been examined for substantial transfer to vegetation. In addition, examination of the distribution of natural radioactivity has been carried out to identify areas of Ireland where levels are well above average.

2. Results.

The analysis of soil samples has shown the presence of Chernobyl fallout and weapons test fallout in all cases. These have been quantified and indicate that Ireland was among the more seriously affected members of the European Community as a result of the Chernobyl accident. All agricultural land tested has shown very low transfers of fallout isotopes to vegetable and cereal crops. However, it has been found that substantial transfer occurs to heather (Erica) growing on poor qaulity or peat soils. This in turn has led to identification of areas in which high levels of caesium could be expected to occur in sheep. Some confirmation of this has been established by examination of meat measurement results from the Irish Nuclear Energy Board. Measurements on milk powder from one area of the country have shown that little artificial radioactivity is now reaching the population via milk consumption. Continuing work on heather honey has confirmed that up to 500 Bq/kg of caesium-137 are present in some samples, much of it resulting from the Chernobyl accident. This work has been hampered by the very low honey crops available in 1987.

Six laboratories participated in an intercalibration exercise organised by this laboratory on a sample of Irish soil containing Chernobyl and weapons test fallout. The agreement between laboratories was excellent and indicates that considerable confidence may be placed on measurements made by the laboratories involved.

The measurement of the natural radioactive series and potassium in soil has identified several areas of high potassium levels and one area where high concentrations of uranium isotopes in soil exist. This area appears to coincide with an area of high radon levels in houses and may extend over some thousands of square kilometres.

Assessment of the population dose has not yet been carried out due to difficulties in obtaining accurate data on quantities of different foodstuffs originating in the different areas of the country.

3. Discussion.

The establishment of very low transfer factors for fallout isotopes to foodstuffs for most agricultural areas is satisfactory. The identification of certain types of low grade soils where transfer is higher than average gives rise to the need for further investigation. The intercalibration exercise proved to be very worthwhile and confirms confidence in the measurement techniques used.

The discovery by soil measurement of an area of high uranium content

which apparently matches an area of high radon levels in houses was unexpected and needs to be looked at in more detail. It is not possible with present information to give a numerical value of the population dose from consumption of Irish foodstuffs. However, better statistical information on the fraction of different foodstuffs coming from different regions should make it possible to give upper limit values.

IV. Objectives for the next reporting period:

Further measurements will 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 factors to heather have been found and transfers to vegetables in such soils will be investigated. A more detailed study will take place in the area of higher than average uranium series concentrations and this will be related to domestic radon levels if possible. Collaboration with other laboratories within the European Community will continue and the relation between radioactivity levels in heather and sheep meat will be explored in association with the national laboratory of the Nuclear Energy Board. Further efforts to determine population doses will be made.

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

Dr. P. Cawse	Harwell Laboratory Oxford, United Kingdom
Dr. R. Kirchmann	CEN-SCK Mol, Belgium.
Dr. J. McLaughlin	Department of Physics University College, Dublin.
Dr. P.A. Colgan	Nuclear Energy Board 4 Clonskeagh Square, Clonskeagh Road, Dublin.

VI. Publications:

"Natural Radioactivity levels in soil in the Republic of Ireland".
I.R. McAulay and D. Moran.
Paper presented to the Fourth International Symposium on the Natural Radiation Environment. Lisbon, December 1987. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-195-E

C.I.E.M.A.T.
Avenida Complutense n° 22
E-28040 Madrid

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 GOBERNING THE TRANSPORT FROM WATER TO THE SEDIMENTS.

Head(s) of project:

E. IRANZO

Scientific staff:

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 coastal marine environment of Southeast 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. Distribution of $^{239}Pu+^{240}Pu$, ^{241}Am and ^{137}Cs in sediment cores.

2. Inventories of $^{239}Pu+^{240}Pu$ in the continental shelf of the area.

3. Distribution of the Heavy metals fraction associated with hydrous Fe and Mn oxide coatings on the surfaces of sediment particles.

4. Relationship between porosity and plutonium concentrations in surface sediments.

5. Sediment depth corrected for compaction in sediment cores.

6. Chronology of sediments by the ^{210}Pb method.

7. Chemical and mineralogical composition of sediments.

III. Progress achieved:

1. METHODOLOGY

In addition to the methods already specified in the 1986-progress report, the procedures used for the new task are described below.

1.1 ^{241}Am and ^{137}Cs measurement

- ^{241}Am analysis.

Americium analysis are performed in the same matrix than plutonium. The americium fraction is purified by sequential separations according to E.Holm Method. Americium was electroplated onto a stainless steel disc and counted by alpha spectrometry. Radiochemical yield was obtained using ^{243}Am as tracer. The reliability of the method was checked against IAEA standards.

- ^{137}Cs analysis.

^{137}Cs measurements are performed by gamma spectrometry using an intrinsec Germanium spectrometer. The accuracy of the measurements was checked with standard samples of sediments from IAEA. Intercalibration exercises confirm the reliability of the data.

1.2 Plutonium inventory in the continental shelf

The inventory was obtained by the following equation

$$\text{Inventory} = \sum_{i=o}^{n} a_i Dw_i / s_i$$

a_i = activity concentration of Pu; Dw_i = dry weight of each slide;

s_i = section of each slide.

1.3 Heavy metals measurements

Analysis of the heavy metals (Cr, Mn, Fe and Pb) associated with hydrous Fe and Mn oxide coatings on the sediments particles have been performed on 1g of sediment using the Rapin's sequential extraction procedure. Measurements have been made by Atomic Absortion.

International intercalibration exercises(ICES and MEDPOL II) confirm the reliability of the data.

1.4 Sediment depth correction

The sediment depth correction (Z') is calculated by the equation:

$$Z' = Z + \int_0^Z (\phi_0 - \phi_z)/(1-\phi_z)\,dz$$

where:

ϕ_0 Porosity of sediment at the interface Z=0

ϕ_z Porosity at any depth, as described in last report.

1.5 Chronology of sediments by the ^{210}Pb method

The ^{210}Pb method has been applied to study the chronology of sediments. The model of Krishnaswami et al has been assumed as a first approach.

Total ^{210}Pb measurements are performed by gamma spectrometry with an intrinsec Germanium detector. The supported ^{210}Pb is obtained by the measurement of ^{214}Pb.

1.6 Chemical-Mineralogycal composition of sediments

The chemical composition has been determined by Plasma and Atomic Absortion Spectrometry on dry sediment samples quartered by a Universal Channel Sampler.

The mineral composition has been calculated by the association grades between the chemical compounds.

2. RESULTS

2.1 Distribution of $^{239}Pu+^{240}Pu$, ^{241}Am and ^{137}Cs concentration in sediment cores

2.1.1 Plutonium

The distribution of plutonium concentration has been determined in six sediment cores (34, 12, 31 30, 18 and 29)and twenty one points of superficial sediments sampled at the continental shelf.

The Pu distribution in the layers is shown in Fig.2. Values of Pu concentration lower than detection limit have not been recorded. The highest concentration values have been found at the core nº 31, located south of the Almanzora river mouth. The cores 12 and 34, located north of the river mouth, show the lowest values.

The variation coefficient of diferences between results of the Eckerd College and CIEMAT is 20%.

2.1.2 Americium.

^{241}Am concentrations in core 12 are in the range of 0.58-0.34 Bq/kg for layers 0 to 7. ^{241}Am over the detection limit has not bee found in the layers under the number 7.

2.1.3 Cesium

^{137}Cs concentrations in core 12 are in the range of 2.9-0.6 Bq/kg for layers 0 to 9. ^{137}Cs over the detection limit has not been found in the layers under the number 9.

2.2 Plutonium inventories

The calculated $^{239}Pu+^{240}Pu$ inventories are:

Core 34 = 177 Bq/m^2	Core 30 = 327 Bq/m^2
Core 12 = 128 "	Core 18 = 254 "
Core 31 - 529 "	Core 29 = 252 "

The $^{239}Pu+^{240}Pu$ inventories from other mediterranean areas are:

Taranto Gulf = 29.6-336 Bq m^2 Ligurian Sea = 111-185 Bq m^2

These values can be compared with our reported inventories.

2.3 Heavy metals distribution

The heavy metals concentration distribution in all of the sediment cores of the continental shelf is similar. The highest values are in the core 34; this core was sampled near the dumping area of a factory. The range of concentration values, except for core 34, are the following:

Mn: 60-80 µg/g	Pb: 20-40 µg/g
Fe: 2-5 mg/g	Cr: 1-2 µg/g

The highest values of heavy metals concentration in superficial sediments of continental shelf are in shipeks 1, 2, 3, and 37; all of them are close to the sampling area of core 34. The range of concentration values are the following:

Mn: 51-990 µg/g	Fe: 1.2-16.9 mg/g
Pb: 5.1-395 µg/g	Cr: 0.7-458 µg/g

2.4 Relation between porosity and plutonium concentration

The Fig.3 shows the relationship between plutonium concentration and porosity in superficial sediments of the continental shelf. These values are distributed in two lines. The linear correlation coefficient is 95% for both. We will try to determine the factors that influence in these distributions. The slope value(line 1) is similar to the ones reported by Jennings and Papucci in the Mediterranean Sea.

2.5 Corrected sediment depth

The sediment depth corrected for compaction are shown in Table 1. Variations between cores are consequence of differences in porosities.

2.6 Chronology of sediments

The maximum concentration of $^{239}Pu+^{240}Pu$ in core 12 looks like correspond to the year 1963 (2.7 cm depth), as must be confirmed from the ^{210}Pb and ^{214}Pb concentrations measured for this and other cores. There has,nevertheless, been some sediment mixing to carry Pu to depths below the 1945 horizon. Physical and bioturbation processes could be implicated in the existence of Pu below the 1945 horizon.

2.7 Chemical-mineralogical composition of sediments

The sediments from all the sampling stations have been analysed. The results show:

- The average composition is SiO_2(35% ± 6 %), CaO(23% ± 4 %), CO_2(20% ± 4 %) and Al_2O_3(8% ± 2 %).

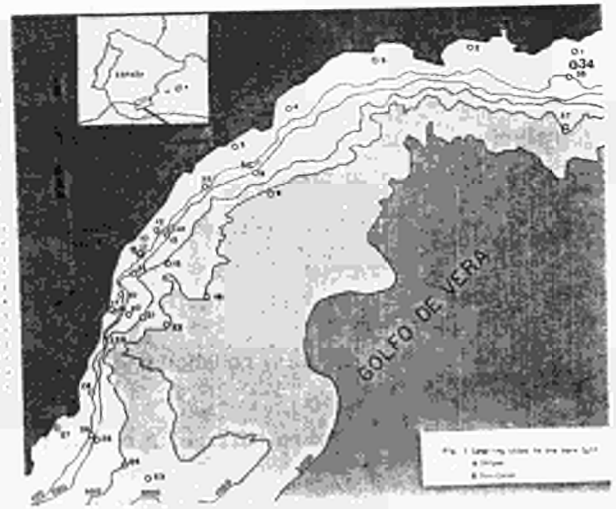
- The mineral compounds, as is shown by the association level, are: Calcite 42%, Illite 29%, Quartz 24% and Dolomite 4%.

- In relation to the silica contents the samples are classified in three groups: G-1(SiO_2 > 48%), G-2(48% > SiO_2 > 20%) and G-3(SiO_2 < 20%).

- The highest contents of silicates correspond to the areas close to the mouths of Almanzora and Antax rivers.

- The highest content of quartz defines the facies of sediments nearest the coast.

- The highest contents of carbonates are at a depth of 100 - 200 m in the proximal area of the continental slope.


GOLFO DE VERA

IV. Objectives for the next reporting period:

- Sampling of sea water to determine the particle populations at the study area.

- To continue ^{239}Pu+^{240}Pu, ^{241}Am, ^{137}Cs, ^{210}Pb and ^{214}Pb analysis.

- To continue Heavy metals analysis by A.A.S.

- To obtain conclusions about the main objectives of the project.

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

C.D.Jennings, Eckerd College, P.O. Box 12560, St Petersburg FL. 33733. USA.

Argeo Rodriguez de León. Instituto Español de Oceanografía. Sor Angela de la Cruz nº 9. 28020-MADRID, Spain

VI. Publications:

L.Romero y C.Gascó."Distribución e inventario del plutonio en sedimentos marinos procedentes del S.E. Español". II. Congreso Nacional de la Sociedad Española de Protección Radiológica. Toledo 4-6 Noviembre de 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor.

Contract no.: BI6-B-056-D

Gesellschaft für Strahlen-
und Umweltforschung mbH
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

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.

BI6-056-D

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 Radiohydrometrie
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:

Further studies of radioiodine conversion resp. sorption in batch and laboratory column tests under variation of soil typ and septic conditions, in order to extend data base.

Installation of outdoor facility, first comparative tests.

Tests on relations between precipitation intensity, soil structure and depth of radioiodine infiltration under consideration of iodine biogeochemistry.

Adaption of tests for enzymatic and bioactivity.

Development and test of a model for simulation of processes of radioiodine conversion and sorption.

III. Progress achieved:

1. Methodology

Inclusion of outdoor experiments.

Further development of application of enzymatic tests and tests on microbial activity.

Improvements in batch shaking and column percolation techniques.

Development of the basis of a model for simulation of radioiodine transformation and fixation processes.

2. Results

The furthermore performed experiments showed that transformation of inorganic iodine (I^-) into organic bond (iodination of organics in environmental systems, in solution as well as in soil solids) generally can be described by first order reaction kinetics. In view of correspondingly running back reactions (de-iodination) equilibria of iodine species distribution are achieved. Aerobic conditions are a prerequisit for iodination reactions, while de-iodination also runs in anaerobic systems.

A compartment model was developed for simulation of radioiodine transformation and fixation processes on the basis of the above insights (Fig. 1). The established fact of increasing sorption strength of iodine in sediments with time of incubation, was considered by installation of two boxes (F_1 and F_2) with different rates and strengths of iodine fixation. Examples of simulations of iodine speciation dynamics are given in Fig. 3 and 4, Fig 3 to compare with experimental results of Fig. 2. Likewise simulations can be run with quite different starting conditions e.g. iodine fully sorbed or in any other distribution.

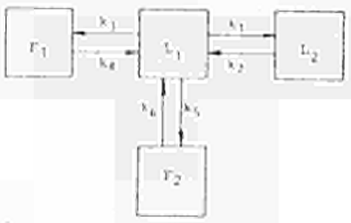


Fig. 1: Compartment Model of iodine conversion and sorption processes in soil/water systems.

L_1 represents the inorganic iodine in the system which is fully in solution as I^-; IO_3^- which will be reduced is not considered

L_2 represents the dissolved iodine in organic bond

F_1 and F_2 represent the iodine bound by solid soil organics

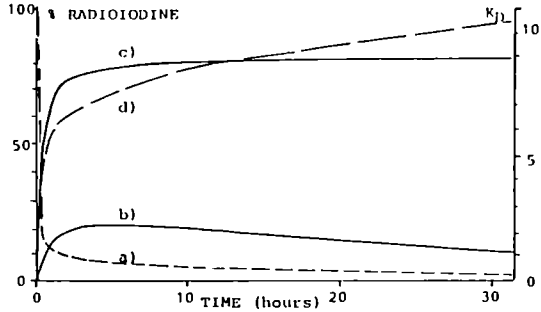


Fig. 2: Dynamics of radioiodine speciation in a soil/water batch experiment (loamy sand). Batch spiked at t=0 with ^{123}I (as I^-).

a) dissolved I^-
b) dissolved organic radioiodine
c) radioiodine bound by soil
d) distribution coefficient (K_D)

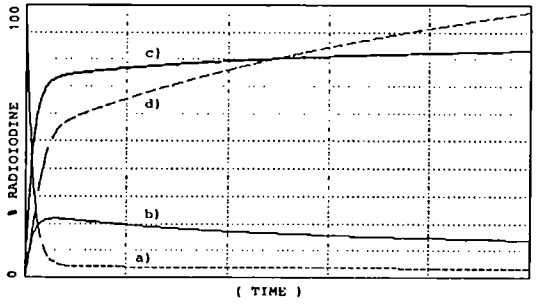


Fig. 3: Dynamics of radioiodine speciation in a soil/water batch as obtained by numerical simulation according model given in Fig. 1, starting with 100 % of radioiodine (as I^-) in solution.

k_1=0.036 k_2=0.009
k_3=0.1 k_4=0.006
k_5=0.012 K_6=0.0001

For a) - d) see Fig. 2

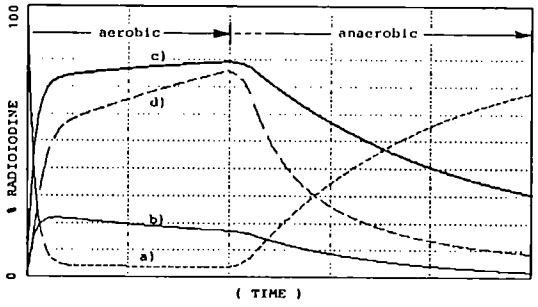


Fig. 4: Dynamics of radioiodine speciation in a soil/water batch by numerical simulation as in Fig. 3, but with change from aerobic to anaerobic conditions by tuning k_1, k_2 and k_3 to zero. This example represents a process which could occur when flooding a soil.

For a) - d) see Fig. 2

3. Discussion

The transport behaviour of radioiodine in terrestrial environments is governed by biogeochemically initiated processes. The type of reactions appears in uniformity (iodination and de-iodination of organics), however, rate and direction of reactions depending on bioactivity and redox conditions of the systems. The uniformity of reactions allowes for application of general modelling of the related processes, however, in detail of this, more information on the influence of variation of different parameters seemes to be needed.

IV. Objectives for the next reporting period:

Characterisation of soils and water in respect to enzymatic and bioactivity.

Batch distribution and column migration tests of radioiodine sorption and migration under consideration of enzymatic and bioactivity.

Influence of hydraulic conditions on radioiodine migration in outdoor experiments.

Further development of modelling of iodine sorption and transport processes, especially in respect to variation of soil/water ratio.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

Tissue and subcellular distribution of technetium in living organisms.

Head(s) of project:

Prof. J. PIERI
Laboratoire de Biochimie et Radiobiochimie - Université de Nantes
Rue de la Houssinière 2 F-44072 NANTES Cedex03

Scientific staff:

Pr. J. PIERI, J. GALEY, F. GOUDARD.

I. Objectives of the project:

L'étude des mécanismes biologiques de rétention, de transport intra-cellulaire et d'élimination de radionucléides présents dans l'environnement marin constitue l'objectif de ce travail.

II. Objectives for the reporting period:

Déterminer les ligands bioorganiques du technétium-95m dans les cellules digestives du homard ; les isoler et les caractériser. Comparer avec les ligands de métaux tels que cadmium, cuivre, zinc et fer.

III. Progress achieved:

Les homards (Homarus gammarus) sont contaminés au laboratoire de Radioécologie marine, CEA La Hague. Un homard constituant le témoin est maintenu dans les mêmes conditions dans une eau non contaminée.

Méthodologie - Des résultats rapportés précédemment montraient la forte accumulation du ^{95m}Tc dans la glande digestive du homard. Notre essai de détermination des molécules liant le technétium a ainsi été effectué sur la glande digestive de ces animaux. Les organites cellulaires sont extraits et purifiés comme il a été précédemment décrit, par centrifugation. La séparation des constituants du cytosol, des membranes plasmiques et des lysosomes est effectuée par chromatographie sur gel et d'échange d'ions. Les métaux stables sont dosés par absorption atomique.

Résultats et discussion - Le technétium est essentiellement associé aux constituants du cytosol (_78 %) dans la glande digestive. Contrairement au transuranien ^{241}Am accumulé dans les lysosomes, cette fraction contient une faible proportion (12 %)de ^{95m}Tc.

Par chromatographie sur gel de Sephacryl S-300 et Séphadex G 75 nous avons établi que le technétium du cytosol est lié à des molécules de faible masse molaire (_12000). Ces molécules possèdent des propriétés ioniques différentes et sont séparées par chromatographie sur résine échangeuse d'ions DE 52 : - 15 % du ^{95m}Tc déposé ne sont pas fixés par la cellulose. - 51 % sont élués en deux pics distincts A et B, par le gradient de concentration de Tris-HCl 10 mM - 400 mM, pH 8,6. Le restant du technétium est élué par le gradient de Tris-HCl 0,4 M - I M, pH 8,6.

L'analyse des métaux Cd, Cu, Fe, Zn en absorption atomique montre que seul le cuivre est élué en un pic superposable à celui du ^{95m}Tc. Le cadmium est élué avec les composés de masse molaire plus élevée _ 150 000 ; le volume d'élution du fer correspond à celui de la ferritine (440 000) et le zinc est distribué en une série de pics entre le volume vide et le volume total de la colonne.

La composition en acides aminés des protéines A et B isolées par chromatographie d'échange d'ions est donnée Tableau I.

Acides aminés	A	B	Acides aminés	A	B
Asx	9,57	10,42	Leu	8,69	6,11
Thr	7,94	6,33	Tyr	0,75	1,54
Ser	8,16	6,21	Phe	1,02	1,54
Glx	11,26	14,57	His	26,39	12,48
Gly	6,43	9,15	Lys	3,11	1,76
Ala	3,96	6,11	Arg	1,31	1,68
Val	3,06	4,02	Pro	3,91	4,40
Met	0,79	1,31	A. Cys	2,94	3,67
Ile	1,72	2,44	Trp	0	0

Tableau 1. Composition en acides aminés des protéines liant le ^{95m}Tc dans la glande digestive du homard Homarus gammarus.

La faible teneur en cystéine, par conséquent en SH ; la présence d'acides aminés aromatiques : tyrosine et phénylalanine ; la forte proportion en histidine:26% et 12% respectivement, font que ces protéines anioniques de poids moléculaire apparent 12 000 ne correspondent pas aux critères de définition des métallothionéines.
La forte teneur en histidine pourrait être reliée à la présence de cuivre dans ces fractions. En effet, le noyau imidazole de l'histidine peut former des complexes avec le cuivre. Par exemple, l'ion Cu(I) est lié à 3 imidazole dans le site actif de l'hémocyanine.
Des complexes de ce type pourraient exister dans ces molécules qui fixent également le technétium.

La solubilisation des lysosomes et des membranes plasmiques en présence de SDS 0,1 % entraine 85 % et 65 % successivement, du ^{95m}Tc dans le surnageant. Le radionucléide ainsi solubilisé est associé à un ensemble de composés de masses molaires très différentes. La seule analyse chromatographique ne permet pas de conclure sur la présence ou non, dans les lysosomes et les membranes plasmiques, de molécules liant le technétium dans le cytosol.

IV. Objectives for the next reporting period

Il s'agira de montrer les effets du stockage de radionucléides contaminants dont nous avons l'intention d'établir les constantes de liaison moléculaires, ce qui nous permettra une évaluation du risque alimentaire et une meilleure connaissance de la radioprotection dans ce domaine.

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:

GOUDARD Françoise : Contribution à l'étude du métabolisme des radionucléides ^{252}Cf, ^{241}Am et ^{95m}Tc. Thése de Doctorat d'Etat, Sciences (1987).

RADIATION PROTECTION PROGRAMME
Progress Report

1987

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. A. Preston
Fisheries Laboratory
Ministry of Agriculture,
Fisheries and Food
GB- Lowestoft, Suffolk NR33 OHT

Telephone number: (0502)62.244

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 R J Pentreath; 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) To analyse and interpret data on the distributions of artificial radionuclides in the sediments and pore waters of the northeast Irish Sea.

(ii) To use fluidized-bed models of sediments with tracers to study the influence of chemical conditions on radionuclide behaviour.

(iii) To investigate the seasonal dependence of radionuclide scavenging using the natural disturbances from equilibrium of $^{234}Th/^{238}U$ and $^{210}Pb/^{226}Ra$.

(iv) To survey and interpret the distribution of the benthic fauna in the northeast Irish Sea.

(v) To continue the development and validation of a multi-box model to describe the behaviour of long-lived radionuclides in the Irish Sea.

III. Progress achieved:

(i) Progress has been made in the analysis and interpretation of the data obtained from CIROLANA cruise 5/83 on the distributions of artificial radionuclides in the sediments of the northeast Irish Sea. The depth distributions of ^{106}Ru and ^{134}Cs have been used to estimate sediment mixing rates assuming it to be a quasi-diffusive process. For ^{106}Ru the values are in the range 3-180 cm^{-2} a^{-1} and are of the same order as those which have been derived from data for the natural radionuclide ^{234}Th. For ^{134}Cs the values are in the range 10-2x10^4 cm^2 a^{-1} and are generally higher and more variable than those for ^{106}Ru.

The variation in the $^{137}Cs/^{134}Cs$ ratio has indicated that there appears to be a time lag between the discharge and the incorporation of the nuclides in the offshore, muddy sediments and that these sediments are mixed more rapidly than the inshore, sandy areas.

Examination of the $^{137}Cs/^{239/240}Pu$ ratio in the discharges and as a function of depth in the seabed appears to indicate that the sediment has become a nett source of ^{137}Cs as the discharges of this nuclide have declined.

(ii) It has been confirmed that the major diagenetic processes occurring in the sediments of the northeast Irish Sea are oxygen consumption, nitrate reduction and iron and manganese reduction. There is no correlation between the distributions of these processes and the distribution of Pu and Am in the sediments. It appears that, despite significant chemical changes to both the sediment particle surface and the interstitial solution, a simple adsorption model may be appropriate for both Pu and Am. Fluidized bed experiments with ^{134}Cs as a tracer have shown that a change from oxic to anoxic conditions has little effect on the adsorption of this nuclide by Irish Sea sediments.

(iii) Samples of water, suspended load and sediment have been collected at a series of stations in the eastern Irish Sea on five occasions during 1986. The $^{234}Th/^{238}U$ and $^{210}Pb/^{226}Ra$ disequilibria are being measured to allow an assessment of the seasonal variation in radionuclide scavenging to the seabed to be made.

(iv) The analysis and interpretation of the surveys of the benthic fauna in the northeastern Irish Sea have been completed and the data prepared for publication.

(v) The development of the multi-box model of radionuclide behaviour in the Irish Sea has continued and a number of improvements made. The overall water circulation pattern over time has been modified to improve the match between the long time series of observations of ^{137}Cs in seawater and the predictions of the model. Water exchange rates between boxes have been adjusted to take account of the results obtained from long-term current meter deployments made in 1986. In addition, the proportions of silt, sand and gravel in the seabed sediment and the suspended load can now be specified for each area of the model.

Simple 1-dimensional models are being used to examine how the effects of bioturbation might be incorporated into the overall model.

IV. Objectives for the next reporting period:

(i) The analysis and interpretation of past data on the distributions of long-lived radionuclides in the sediments of the northeast 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 northeast 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.

V. Other research group(s) collaborating actively on this project [name(s) and address(es)]: None.

VI. Publications:

Kershaw, P., Young, A. Scavenging of ^{234}Th in the eastern Irish Sea. J. Environ. Radioactivity 6 (1988) 1-23.

Pentreath, R. J. The interaction with suspended and settled sedimentary materials of long-lived radionuclides discharged into United Kingdom coastal waters. Continental Shelf Res. 7 (1987) 1457-1469.

Gurbutt, P. A., Kershaw, P. J. Biological mixing of shelf seas sediments with implications for modelling. ICES CM 1987/C:22 (13 pp, mimeo).

Kershaw, P. J., Pentreath, R. J., Harvey, B. R., Lovett, M. B., Boggis, S. J. Apparent distribution coefficients of transuranium elements in UK coastal waters. In: Application of Distribution Coefficients to Radiological Assessment Models. (Sibley, T. H. and Myttenaere, C., Eds.) Elsevier Applied Science Publishers, London, 277-287.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-048-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 OX 11 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
Mr. G.J. Ham

I. Objectives of the project:

To investigate the time dependent transfer of Pu-239, Am-241, Sr-90 and Cs-137 into food crops from soils of widely differing textural classes which are representative of those found in the EEC.

II. Objectives for the reporting period:

(i) To investigate the effect of glyphosate, the active ingredient in the widely used herbicide 'Tumbleweed'/Round Up' on the uptake of radionuclides into peas and carrots from peat, sand, loam and clay soils.

(ii) To investigate the time dependent transfer of radionuclides into winter barley from soils radio-labelled in 1984.

(iii) To determine radionuclide concentrations in soil water under field conditions in order to more accurately assess radionuclide availability to crops.

(iv) To complete the analysis of 1985 crops and soils for strontium.

III. Progress achieved:

Methodology

(i) Sand, loam, clay and peat soils were labelled with Pu-239, Am-241, Sr-90 and Cs-137 in June 1987 and placed in 281 tubs (26x29x40). Glyphosate was applied at a standard agricultural rate (3 l/ha) to one of each pair of tubs, before planting with peas and carrots. Samples from the harvested crop and associated soils are currently undergoing radiochemical analysis so that transfer factors for glyphosate treated and untreated soils can be calculated.

(ii) In October 1987 winter barley was sown in large tubs containing previously radio-labelled peat, sand, clay and loam. This crop will be harvested in Summer 1988 and the transfer factors to grain compared to those obtained for winter barley from the 1986 harvest.

(iii) Soil water is being collected from several sites in Cumbria and Lancashire using two different methodologies. The first uses an immiscible liquid displacement/high speed centrifugation technique on soil brought back to the laboratory from the field. The second, non-destructive method uses porous ceramic pots installed at various depths in a range of agricultural soil types. This latter technique enables soil water chemistry to be routinely collected and monitored throughout the year. Porous pots have also been installed in the large tubs described in Part 1b above to provide supplementary data on uptake of radionuclides into the barley crop.

(iv) The strontium analysis of the 1985 crops and soils are now completed and the results are presented below.

Results

1985 CEC Soil Sr Values

Tub No.	Soil	Bq/Kg dry Wt. Spring	Autumn	Mean
1	Loam	1681 ± 67	1818 ± 72	1749
2	Loam	1641 ± 71	1826 ± 72	1734
3	Peat	6152 ± 242	6888 ± 269	6520
4	Peat	10932 ± 426	8440 ± 330	9686
5	Sand	1736 ± 73	1674 ± 67	1705
6	Sand	2094 ± 85	1678 ± 67	1886
7	Clay	2752 ± 109	716 ± 29	1734

1985 CEC Crop Sr Results

Description (tub no.)	Weights Wet	Dry	Bq/Kg Dry	Bq/kg Wet	Wet Crop/T.F. Dry soil
Beans Loam (1)	707.6	88.6	1931 ± 75	242 ± 9	0.1382
Beans Peat (3)	1353.2	153.6	737 ± 29	84 ± 3	0.0128
Beans Sand (5)	1846.2	189.6	3145 ± 122	323 ± 13	0.1894
Beans Clay (7)	628.1	109.5	925 ± 36	161 ± 6	0.0586
Cabbage Loam (1)	3664	379.1	6640 ± 272	687 ± 28	0.3928
Cabbage Peat (3)	6391	629	2508 ± 98	247 ± 10	0.0379
Cabbage Sand (5)	4515	472.7	10775 ± 429	1128 ± 45	0.6616
Cabbage Clay (7)	2027	204.6	3942 ± 157	398 ± 16	0.1446

Discussion

The soil strontium results show reasonable agreement between the spring and autumn samples for all tubs except the clay soil (tub 7). The mean activity has therefore been used to calculate the transfer factors for all soils except the clay where the spring result was used.

The ratio of the transfer factors from the different soil types for the same crops are:-

Relative Transfer Factors (Loam soil = 1)

Soil Type	Relative T.F. Beans	Cabbage
Loam	1.0	1.0
Peat	0.092	0.096
Sand	1.4	1.7
Clay	0.42	0.36

These numbers suggest there is a constant effect on the transfer factor in the two crops, due to the soil type.

IV. Objectives for the next reporting period:

(i) To determine radionuclide concentrations in soil water under field conditions in order to more accurately assess radionuclide availability to crops.

(ii) To continue the investigation of the effect of glyphosate and other farming practices on the uptake of radionuclides into peas and carrots from peat, sand, loam and clay soils.

(iii) To investigate the time dependent transfer of ^{239}Pu, ^{241}Am, ^{90}Sr, ^{137}Cs into winter barley from soils radio-labelled in 1984.

(iv) To complete the analysis of 1985 and 1986 crops and soils for ^{90}Sr.

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

VI. Publications:

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. Popplewell

Scientific staff:

Dr. R.A. Bulman
Dr. G.M. Clint
Mr. G. Ham
Dr. J.D. Harrison
Dr. G.P.L. Naylor

I. Objectives of the project:

The objectives of the project are to investigate the chemical forms of radionuclide contaminants of foodstuffs, and the bearing that these have on their gastrointestinal uptake.

II. Objectives for the reporting period:

The objectives for the reporting period were :-

(i) Continue studies of the chemical form of actinides in foodstuffs.

(ii) Characterise physico-chemical and biological factors that may influence the absorption of ingested radionuclides.

(iii) Measure the absorption of niobium in adult and newborn guinea pig.

(iv) Measure the absorption of Pu and Am from in vitro labelled potato in a primate species, the marmoset.

III Progress achieved.

i) Whereas much research has been directed at investigating the mechanism by which low molecular weight complexes bring about the uptake of actinide cations, the role of macromolecules in the uptake has been the subject of less research. It cannot be excluded that actinide cations bound to proteins might be taken across the gut wall by a transport process which is selective for a specific peptide sequence. As liver has sometime been used as a source of biologically incorporated Pu and Am excised livers, as well as kidneys, from animals injected with ultra filtered citrates of U, Pu and Am, were cooked, subjected to a simulation of human digestion and the chemical form in the digest examined by size exclusion chromatography on Sephadex G25 superfine. Radiochemical analysis of the eluates from liver showed that ^{239}Pu and ^{241}Am were bound by an iron-rich macromolecule, possibly ferritin, and that there was no radioactivity associated with low molecular weight fractions. The soluble ^{239}Pu released from kidneys was equally distributed between two high molecular weight components, whereas both ^{233}U and ^{241}Am were each associated with only one high molecular weight substance. The identity of these fractions is not yet established. A series of studies are also in progress to characterise the degradation products of in vitro-lablled ^{239}Pu-ferritin. The radiolabelled macromolecular form originating from liver is currently being characterised and will be used in feeding experiments with laboratory animals.

Investigation of the influence of the digestive process on the speciation of ^{239}Pu in skimmed milk has shown that the radionuclide, initially added as the citrate, can be isolated as a high molecular weight material after the digestion process. Examination of the molecular weight distribution of ^{239}Pu in the simulated digestate of ^{239}Pu-lactoferrin, initially labelled by adding the citrate and then dialysing the mixture overnight against running distilled water, showed the radionuclide to be almost equally distributed between two high molecular weight components. These chemical forms of ^{239}Pu originating from digested milk and lactoferrin are being characterised and will also be used in laboratory feeding experiments.

The low level of uptake of actinides into plants is well recognised and a procedure for producing increased uptake would simplify research into the speciation of actinides in plants. Techniques used in plant-ion biophysics for studying the uptake of essential trace elements have been evaluated for actinide studies. Essentially this cation-loading procedure used thin discs (thickness 1mm, diameter 8mm) of root storage tissue maintained under high aeration in a dilute nutrient solution to which the actinides were added either as the citrates or as the nitrates. Prior to loading the cells with the actinides the discs were maintained for 48h in aerated distilled water regularly changed at 12h intervals. Radiochemical analyses of the discs showed that the uptake of ^{239}Pu, ^{241}Am and ^{233}Pu was almost exclusively into the cell wall region. As there was no significant uptake of radioactivity into the cytoplasm of the storage cells this procedure is of no value for producing cells containing high levels of intracellularly incorporated actinides. The highest levels of uptake were from solutions to which the actinides were added as the nitrates. This uptake phenomenon perhaps points to an accumulation of radioactivity as polymeric species.

The production of potato tubers radiolabelled with ^{239}Pu and ^{241}Am has been previously reported. Evidence for a biological incorporation of these

radionuclides into potatoes can be inferred from the transfer, 2-5%, of the radionuclides from the tubers into new shoots of approximately 25cm length.

Electroultrafiltration is a procedure which was thought might be of value in our speciation studies. However, only very low levels of ^{239}Pu, ^{241}Am, ^{90}Sr and ^{137}Cs could be mobilized from a variety of soils, consequently the procedure is of no further value for investigations of the speciation of actinides and other polyvalent cations in soils.

(ii) Measurements have been made of the transfer of americium and plutonium in rat milk. Rats with litters aged 1, 5, 10 and 15 days were given intravenous injections of either ^{241}Am citrate or ^{238}Pu citrate and the transfer to the pups over 5 days was determined, taking account of changes in milk consumption and milk concentration of the actinides. The measurements made have been used to estimate total gut transfer in the neonates. Similar results were obtained for Pu and Am and the values suggest that ingestion in milk may increase absorption throughout the suckling period and particularly maintain high levels of about 0.5% towards the time of weaning.

(iii) A study of carrier-free ^{95}Nb absorption in guinea-pigs is now complete. This was undertaken because of the possibility that absorption may be as high as 60% for dietary forms of the element, as indicated by the data included in ICRP 23. For one group of adult animals, ^{95}Nb in a citrate medium was added to milk and administered orally midway though a period of 48 hours during which drinking water was replaced with milk; pelletted food was available at all times. A second group of adults was fasted for 24 hours before the administration of ^{95}Nb in citrate; water was available at all times. Two day old guinea pigs were given ^{95}Nb orally and kept with their mothers. The results for fed and fasted adults and for 2 day old neonates were about 0.8%, 1.4% and 1.5%, respectively. These results support the ICRP 30 value of 1% for adults and the recommendation in a forthcoming NEA Task Group report that the value for newborn children should be 2%.

iv) The gastrointestinal absorption of plutonium, americium and neptunium has been measured in a primate species, the marmoset. The original intention was to administer Pu and Am in potato powder labelled in vivo either by foliar application or by injection into the original tuber. However, it did not prove possible to label potato with a sufficiently high concentration for a single administration to marmosets. Instead, the in vivo labelled potato was fed to rats and hamsters as described in last years report and marmosets were given in vitro labelled potato powder. The absorption of Pu administered as the citrate complex was also measured and the absorption of Np was measured only as the citrate. Results obtained were; for plutonium, averages of about 0.14% for ^{239}Pu administered as the citrate complex and 0.24% for ^{239}Pu in potato; for americium, a value of 0.06% after administration in potato; and for neptunium, 0.18% for ^{237}Np as the citrate complex. Because intestinal phytase may be involved in the release of Pu and possibly Am from phytate in potato, the level of the enzyme in marmosets was assayed. The value obtained of 0.13 units/mg protein at a pH optimum of 7.5 is similar to the level in the rat. Levels in human intestine are low. In this respect the marmoset would appear not to be a good model for man.

A human volunteer experiment to measure the absorption of ^{239}Np and ^{242}Cm has been started and will continue in 1988. The first subject has been given intravenous injections of ^{239}Np and ^{242}Cm citrate solution; an

oral dose will follow within the next few weeks. Urinary excretion after intravenous injection was similar to that observed previously in the rat. The cumulative excretion over the first day was 29.8% for ^{239}Np and 5.6% for ^{242}Cm. Over the following 6 days it was 10.3% and 3.5% for ^{239}Np and ^{242}Cm respectively.

Past work on hamsters has, in separate absorption experiments with americium and curium, suggested that there may be some differences between them in their behaviour in the body. A study is in progress to measure americium and curium absorption in guinea pigs from a mixture of these radioelements.

IV. Objectives for the next reporting period:

The objectives for the next year are to:-

(i) continue studies of the chemical form of actinides in foods, including the form of Pu in milk.

(ii) continue with the human volunteer experiments to measure the absorption of Np and Cm administered as the citrate complexes.

(iii) conduct animal experiments to compare the effects of chemical form on the absorption of Am and Cm

(iv) continue the development of analytical biochemical techniques to characterise both high and low molecular weight binding species for actinides.

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

VI. Publications:

Comments on the paper by G.J. Hunt, D.R.P. Leonard and M.B. Lovett, "Transfer of environmental plutonium and americium across the human gut". J.D. Harrison, H. Smith and J.W. Stather. Sci. Total Environ. 64, 325-329 (1987).

The effect of ingested mass on plutonium absorption in the rat. J.D. Harrison and A.J. David. Health Phys. 53, 187-189 (1987).

The gastrointestinal absorption of plutonium and americium in neonatal mammals. In: Age-related Factors in Radionuclide Metabolism and Dosimetry. Proc. CEC Workshop, Angers, France, November 1986. pp27-33 (1987).

Plutonium and americium uptake in rats fed with Cumbrian shellfish - Implications for estimates of dose to man. J.D. Harrison, H. Smith and A.J. David. Sci. Total Environ. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Landbouwhogeschool
Agricultural University
Salverdaplein 10
NL- Wageningen

Contract no : BI6-B-051-NL

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

Dr. J. van den Hoek
Dept. Dierfysiologie
Landbouwhogeschool
Haarweg 10
NL- Wageningen

Telephone number: 83703/83023

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, Dr. V.V.A.M. Schreurs
M.H.J. van den Hoek- ten Have, J.E. Vogt, 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:

The objectives for the reporting period have been to study the incorporation of carbon-14 in organic milk constituents of a lactating cow after the oral intake of carbon-14 containing corn for several weeks. The decrease of carbon-14 levels in milk constituents was followed for over a year after the oral carbon-14 intake had been discontinued.

III Progress achieved:

1. Methodology

Young growing corn plants were exposed to $^{14}CO_2$ for 8 hours. After harvest, the corn was cut, thoroughly mixed and fed to a lactating cow for a period of 33 days. The average daily activity ingested was about 105.6 µCi of ^{14}C. Milk was sampled daily at first and once a week later. Total sampling time lasted for just over 500 days. The different organic milk fractions were separated from the whole milk by standard procedures. Carbon-14 activity was determined by liquid scintillation counting in whole milk, casein, milk fat and lactose.

2. Results

The results are presented in Table 1 which shows the ^{14}C activities in casein, milk fat and lactose during the period when plateau levels in milk were reached (day 6 - day 33). Data on carbon-14 activity during the entire sampling period are available but they are not represented in this report for reasons of space. As one would expect, plateau levels of carbon-14 in milk were obtained quite rapidly, and they were reached in about 6 days in casein, milk fat and lactose. There was a correspondingly rapid decrease of ^{14}C activity in these organic compounds after the cessation of ^{14}C administration. After a few weeks, pools of much slower turnover became visible.

3. Discussion

The carbon-14 activity levels in Table 1 are expressed as concentration levels and as specific activities. The much higher ^{14}C levels in milk fat reflect the higher carbon content of fat as compared to that in casein and lactose. The specific activity (S.A.) value for lactose is rather high as compared to those in casein and lactose. S.A. values in corn were not determined, and for this reason, the S.A. values in organic milk constituents could not be related to the S.A. values of the precursors in corn from which they were formed. It is not possible to make any definite statements on the validity of the S.A. concept under these experimental conditions without this information.
The transfer of carbon-14 from feed to cow's milk, expressed as the percentage of the daily ingested ^{14}C which is secreted in one liter of milk, was as follows: 0.13 for casein, 0.24 for milk fat and 0.18 for lactose. Carbon-14 was being incorporated into casein and milk fat for at least one year after cessation of dosing. The levels in milk fat showed fairly large variations, and an increased rate of mobilization of ^{14}C containing milk fat precursors from body reserves could be seen to occur regularly. It was particularly noteworthy during the first six weeks of lactation after the birth of the calf. The ^{14}C levels in casein were decreasing much more steadily. The results of a regression analysis showed the ^{14}C levels in organic milk constituents to decrease according to a two component half life system. The half life values for the first and rapid component were about 13 hours for lactose and 24 hours for milk fat and casein. The long component was quite insignificant quantitatively for lactose, and about 100 days for casein and milk fat. It may be concluded that only carbon-14 retention in protein and fat merits attention from a radiobiological point of view.

IV. Objectives for the next reporting period:

The transfer and incorporation of tritiated protein, introduced into the abomasum directly through a fistula, into newly synthesized proteins will be studied. This will produce quantitative data on the efficiency of transfer and incorporation of orally ingested tritiated proteins in monogastric animals. The collection and analysis of experimental data on OBT metabolism in growing animals will be completed. The characterisation of the pools of slow turnover for tritiated body fat and tritiated protein in adult animals on the basis of ^{3}H levels in milk fat and milk casein will be continued.

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
Dr. Y. Belot, Institut de Protection et de Sûrete Nucléaire, C.E.N. - F.A.R., B.P. 6, 92265, Fontenay-aux-Roses, France

VI. Publications:

Van den Hoek, J.
The Transfer of Radionuclides to Domestic Animals
Invited Paper on the International Scientific Seminar on Foodstuffs Intervention Levels following a Nuclear Accident, Luxemburg, 27-30 April 1987, EUR 11232, 373-380.

	Carbon-14 Levels	
	pCi/g	S.A.
Casein	4076	7745
Milk Fat	6333	8423
Lactose	4185	9960

Table 1. Carbon-14 levels in casein, milk fat and lactose in cow's milk after continuous ingestion of carbon-14 labelled corn. The data are averages for the period of apparent steady state conditions (day 6 - day 33).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Institut Royal des Sciences
Naturelles de Belgique, IRSNB
Rue Vautier 29
B-1040 Bruxelles

Contract no.: BI6-B-049-B

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 the behaviour of technetium in the marine environment of the Belgian coast.

Head(s) of project:
Prof. D. van der BEN
chef du Département de Biologie à l'I.R.Sc.N.B.
29, rue Vautier B-1040 BRUXELLES

Scientific staff: Four teams:
1.S. BONOTTO, F. CAPOT (C.E.N./S.C.K. - Mol),
2.B. MANIA, Z. MOUREAU, S. WARTEL (I.R.Sc.N.B. - Brussels),
3.F. AUVRAY, M. COGNEAU, K. FONSNY, L. PIGNOLET (Univ. Cathol. de Louvain),
4.J.M. BOUQUEGNEAU, P. GERVAIS, M. LICOT (Univ. de Liège).

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:

Tc uptake by bacteria, protozoa, algae, molluscs and sediments: uptake through food chains, localization in the organisms, binding to organic molecules; influence of light and temperature, toxic effect; modelling of Tc accumulation by the mussel; calculation of Tc distribution coefficients for a sediment rich in organic matter.

III. Progress achieved:

1. METHODOLOGY

1.1. Bacteria and Ciliates
- Autoradiography of Ciliates (Uronema marinum) fed with Tc labelled bacteria;
- Affinity radiography in order to verify whether Tc is bound by bacterial polysaccharides: preliminary experiments.

1.2. Algae
- Radiochemical Tc analyses of brown algae (Fucus spiralis) collected along the Belgian coast;
- Gamma spectrometry, autoradiography, selected extraction, column chromatography and polyacrylamide gel electrophoresis of contaminated algae.

1.3. Molluscs
- In order to make a model of Tc accumulation by the mussel Mytilus edulis, the uptake of Tc^{-99} as pertechnetate ($TcO4^-$) by Phaeodactylum tricornutum BOLIN has been studied.

1.1. Sediments rich in organic matter
- The sediment was deposited in microcosms and the added sea water contaminated with Tc. The microcosms were shaken or not. Mussels were then introduced in the microcosms.

2. RESULTS

2.1. Bacteria and Ciliates

The results of the preliminary experiments indicate that it should be possible to detect the presence of Tc on bacterial polysaccharides by chromatography, and to locate Tc in the Protozoa cell by autoradiography. Further investigation is necessary.

2.2. Algae

Tc uptake by marine organisms varies considerably in different species. The highest CFs were found in some species of brown algae (Ascophyllum nodosum, Fucus serratus, F. spiralis, F. vesiculosus). Under laboratory conditions, the CFs were lower (up to $12x10^1$ in F. serratus) than in nature (up to $5x10^4$ in F. spiralis).

Autoradiographic techniques have allowed to determine the localization of ^{95m}Tc in the brown algae Ascophylum nodosum, F. spiralis and F. vesiculosus and in the red alga Porphyra umbilicalis. The autoradiographs clearly showed an heterogeneous distribution of ^{95m}Tc in the four investigated algae. In Ascophylum nodosum the apical regions of the lateral branches were more heavily labelled than in other parts of the plant. In addition, in F. spiralis, the apical parts with fertile receptacles presented an important Tc accumulation. Similarly, in F. vesiculosus some apices attaining the reproductive stage, were heavily labelled. Moreover, growing vegetative apical parts have accumulated more Tc than the older ones.

Our analyses have shown that Tc is metabolized by the algae where it binds to organic molecules.

Tc uptake by marine algae was influenced by environmental factors, such as light and darkness and temperature. Besides, heat inactivation of the algae (F. serratus) almost completely inhibited Tc uptake. Preliminary experiments have shown that ^{99}Tc causes toxic effects in the unicellular alga Acetabularia, at concentrations higher than 10 $\mu g ml^{-1}$.

2.3. Molluscs

The initial rate of uptake was fast but an equilibrium was rapidly reached and was not significantly different for living and heat-killed cells. This suggests that most of the Tc accumulation results from adsorption processes. The concentrations at equilibrium are about 390 ng Tc g^{-1} for a Tc concentration of 15 ng g^{-1} in the medium. Older cultures have their Tc concentration reduced by half and the wet weight concentration factors calculated for cultures in different conditions of incubation and growth never exceeded 3.

2.4. Sediments

The desorption of the Tc retained by the sediments was very low and the Kd value at the end of the desorption period was of the order of 10^2 for all treatments.

Transfer factors were calculated daily during 27 days (Table 1).

Table 1

Transfer factors between contaminated substrates and MUSSELS (cpm/g fresh weight/cpm/ml/water)

Contaminated source	Experimental conditions	
	shaken	not shaken
Sediments	3.6 ± 0.53	2.50 ± 0.09
Water	0.80 ± -	0.83 ± 0.13
Water-sediments	1.38 ± 0.009	0.97 ± 0.04

3. DISCUSSION

Some species of brown algae strongly accumulate Tc. These large marine plants which have an important biomass, might thus constitute an important link in the transport of Tc through the food chain. In this context, it would be of interest to study the transfer of ^{95m}Tc from the algae to marine animals which feed on them.

Our investigations suggest that Tc uptake by large brown algae is related to cell metabolic activity. The use of specific inhibitors of cell transport and of photosynthesis might further support the hypothesis of an active uptake mechanism.

On the contrary, Tc uptake by Phaeodactylum tricornutum seems to result mainly from adsorption processes.

Concerning sediments, the results show that:

- the data obtained confirm the previous results (Report 86),
- the highest values are obtained for sediments covered with non contaminated water and in shaken conditions.

These results seem to confirm the role of the resuspension of the contaminated surface layer in the contamination of the mussels.

To summarize, it may be said that the mechanical resuspension of the sediments (action of water or mussels) is a factor which has to be taken into account in the contamination of filtering molluscs.

IV. Objectives for the next reporting period:

Research on uptake and distribution of Tc by marine algae, under both laboratory and natural conditions, shall be continued and probably extended to other marine organisms growing along the Belgian coast. The toxic effects of ^{99}Tc at the cellular level shall be further investigated by using unicellular marine algae. In addition, experiments shall be carried out on the effect of the main biological, physical and chemical factors on the process of Tc accumulation in brown algae.
Modelling of Tc behaviour through North Sea food chains should be continued and, if possible, intensified.

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

VI. Publications:

1. S. Bonotto, V. Robbrecht, G. Nuyts, M. Cogneau and D. van der Ben. Marine Pollution Bulletin. (in press)
2. S. Bonotto and D. van der Ben. Boll. Soc. Ital. Ecol., 8, 133 (1987).
3. D. van der Ben, V. Robbrecht, F. Capot, G. Nuyts, A. Bossus, M. Cogneau and S. Bonotto. Arch. Int. Physiol. Biochim., 25, 27 (1987).
4. G. Arapis, S. Bonotto, A. Bossus, G. Nuyts, G.B. Gerber, R. Kirchmann, J. Colard, P. Mathot and M. Cogneau. In: Ocean Processes in Marine Pollution. Vol.1., J.M. Capuzzo and D.R. Kester, eds., Robert E. Krieger Publ. Co., Malabar, Florida, pp. 133-144 (1987).
5. S. Bonotto, D. van der Ben, F. Capot, J.M. Bouquegneau and M. Cogneau. International Symposium on Metals in Coastal Environments of Latin America. Rio de Janeiro, Brazil, August 3-8, 1986 (in press).
6. S. Bonotto, F. Capot, L. Pignolet, J.M. Bouquegneau, M. Cogneau, Z. Moureau, B. Mania and D. van der Ben. Behaviour of Technetium in Terrestrial and Aquatic Environs. Seattle, U.S.A., May 5-10, 1986 (in press).
7. L. Pignolet, F. Auvray, K. Fonsny, F. Capot and Z. Moureau. CCE-DOE joint meeting Seattle to be published in Health Physics 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 of transuranics in aquatic ecosystems.

Head(s) of project:

O. Vanderborght

Scientific staff:

J. Vangenechten
S. Van Puymbroeck
O. Vanderborght

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 role of the hepatopancreas in crustaceans (esp. Astacus leptodactylus) for uptake and storage of transuranics will be assessed together with the subcellular distribution in target organs. It 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 turnover rates will be possible.

II. Objectives for the reporting period:

To study the uptake and the organ distribution of ^{244}Cm in freshwater animals, taking into account the already obtained knowledge on ^{241}Am metabolism.

III. Progress achieved:

Experiments were performed to compare the uptake of ^{241}Am from water in freshwater animals (crayfish : Astacus leptodactylus) and in marine animals (lobster : Carcinus maenas). ^{241}Am was also injected in the stomach of these latter two species to study the subcellular distribution in the hepatopancreas. The analysis and the compilation of these data is still in progress.

The uptake of ^{244}Cm in different freshwater animals was studied. Special attention was focussed on the role of natural organic acids for the speciation of the radionuclide (see project nr 2) and for the uptake by the animals.

Accumulation of ^{244}Cm in the freshwater snail Lymnaea stagnalis yielded concentration factors (CF = radioactivity.kg^{-1} animal wet weight/radioactivity.l^{-1} water) around 300. When natural organic acids were present (10 mg/l), CF values were significantly lower e.g. around 200. This lower uptake in water where ^{244}Cm is bound to organic acids was further reflected in the CF values for the shell (140 vs 230) and for the hepatopancreas (3850 vs 6850).

In clear contrast with former data on ^{241}Am, ^{244}Cm shows high assimilation in hepatopancreas tissue as illustrated by high concentration factors (CF = 1000 to 10000). Whereas ^{241}Am is known to be adsorbed to external structures (shells, carapaces, etc.), ^{244}Cm is found for the largest part in internal tissues. This finding may have important radioecological consequences, and therefore the assimilation of ^{244}Cm in cells and tissues will be an important research item in the next year of study.

We recently started to measure ^{244}Cm bioavailability in rainbow trout Salmo gairdneri. For reasons of safety special experimental tanks had to be built in which ^{244}Cm uptake can be followed in one fish at a time. Also in these experiments we focuss our attention on the influence of organic acids in the water for the biological uptake and organ distribution.

IV. Objectives for the next reporting period:

a. To study the ^{244}Cm uptake in freshwater animals e.g. crayfish and fish.
b. To study the organ and subcellular distribution of ^{244}Cm in freshwater animals.
c. To compare the role of the hepatopancreas in crustaceans (especially in the crayfish Astacus leptodactylus) for uptake and storage of transuranics with the liver in fishes.

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

Prof. J. Pieri, Lab. de Biochemie, Univ. de Nantes, Nantes (France)
Prof. K. Simkiss, Dept. of Zoology, Univ. of Reading, Reading (U.K.)

VI. Publications:

J. Bierkens, J.H.D. Vangenechten, S. Van Puymbroeck, O.L.J. Vanderborght. 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, 15-19 September 1986.

J. Bierkens, 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.

Title of the project no.:

Speciation of transuranics in aquatic environments

Head(s) of project:

D. Vanderborght

Scientific staff:

J. Vangenechten
S. Van Puymbroeck
D. Vanderborght

I. Objectives of the project:

This project aims to correlate the chemical behaviour of the nuclides (^{241}Am + ^{242}Cm) in freshwater environments with their bioaccumulation. This relation indeed remains uncertain although bioaccumulation of 241-Am can differ by one order of magnitude according to the water used. The behaviour of the transuranium nuclide 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 (heavy) 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 adsorption behaviour of ^{242}Cm in sediment-freshwater systems.
B. Study of the speciation of ^{244}Cm (particulate ^{244}Cm fraction and the organically complexed fraction) in freshwater.

III. Progress achieved:

In former experiments, the relation between the speciation of ^{241}Am and its biological uptake was studied. Water quality characteristics which determined the ^{241}Am speciation appeared to be pH, HCO_3, CO_3 and natural organic acids.

We recently studied the adsorption behaviour of ^{242}Cm under laboratory conditions in sediment freshwater systems. The sediments were collected from different freshwater ponds. The adsorption of added ^{242}Cm on the sediments was measured and followed in time. Partition coefficients (Kd = radioactivity per kg of sediment/radioactivity per liter water) ranged from 25 to about 2500.

The results indicate that curium adsorbs strongly to suspended sediments. However one sediment-type displayed a low adsorption (Kd = 25), thus illustrating a high portion of Cm remaining in solution.

Further experiments were carried out to study the relation between the bioavailability of curium and its speciation in the freshwater (see also project nr 1). The particulate curium fraction depended strongly on the amount of natural organic acids in solution. These acids were isolated from a natural boglake water by ultrafiltration. When synthetically prepared water without natural acid was used, up to around 70% of the curium was present in a particulate (> 0.45 µm) form. When natural acids were added at a concentration of 10 mg/l, only around 20% of the curium was present in a particulate form.

Techniques are tested to determined the amount of curium that is associated with the natural organic acids : therefore gel filtration chromatography (Sephadex) is used in the same way as has been done for americium (Vangenechten et al., 1987). Some modifications of the methodology appear to be necessary especially with regard to the cleaning of the gel after each chromatographic run.

IV. Objectives for the next reporting period:

During the next period, the speciation of ^{244}Cm in freshwaters will be further studied. The relation between the physico-chemical characteristics of the water (pH, DOC content, etc.) and the ^{244}Cm species which are measured will be examined.

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.H.D. Vangenechten, N.A. Chughtai, J. Bierkens, O.L.J. Vanderborght. 1987. Similarity of 241-Am and 59-Fe speciation in selected freshwaters and of their adsorption on crayfish exoskeleton. J. Env. Radioactivity, 5, 275-286.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-B-052-B

Union Internationale des
Radioécologistes, UIR
Association Internationale
Rue Cardinal Cardijn 5
B-4480 Oupeye

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 : L.Foulquier, M.Frissel,G.Linsley, C.Myttenaere,A.Paschoa,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. The working group had its second meeting at Merlewood Research Station, Grange-over-Sands, U.K. For details, see under III. "Progress achieved".

b) Radioecological Assessment Working Group

The activities of the new Working Group aim at having better knowledge of the transfer of the radionuclides released in accidental conditions. To that end a first meeting is scheduled in Cadarache :(Mars 1988). It will meet radioecologists from different countries of the world and its scope will be to define the new orientation which has to be given to radioecology in the next ten years.

Moreover, the UIR will participate as an observer to the Co-ordinated Research Programme (CRP) of the IAEA on the

validation of models for the transfer of radionuclides in terrestrial, urban and aquatic environments and to the "Advisory Group meeting of the Agency on the effects of ionizing radiation on terrestrial and freshwater aquatic ecosystems". The IUR has also been charged of the french translation of the SCOPE 25 (vol.I and II) Book on "Environmental consequences of nuclear war".
c) The aim of the working group "Soil-to-Plant Transfer Factors" is to derive reliable transfer factors of radionuclide for nuclear safety assessment studies.

III. Progress achieved:

a) The working group "Soil-to-Plant Transfer Factors" met at Egham, UK, 14-16 April. A summary of all available data, expected values, uncertainties etc. was presented by the chairman of the working group, M.J.Frissel. There is a distinct progress. In the first years the working group concentrated on determining transfer values and to derive correlations with environmental factors as pH, organic matter content, soil type, etc. Gradually the main activities are shiftiong towards the determination of the uncertainty of the expected values. Other lectures were presented (see VI Publications).
On special sessions the following topics were discussed:
Topic 1. Countermeasures.
Topic 2. Direct contamination.
Topic 3. The importance of radionuclide concentration in soil solution.
Topic 4. The meaning of the 95 percent confidence intervals.
Topic 5. The large uncertainty factors for transuranes.
Topic 6. Transfer to other crops and natural ecosystems.
Topic 7. Transfer of the compiled results from producer to user.
A report of the workshop (164 pages) has appeared at the end of 1987. The report includes: Aims of the working group; Recommendations for uptake experiments; Lectures presented; Reports on topic discussed; Listing of all available transfer values.
b) The working group "Plant to Animal Transfer" has a very successful meeting at Merlewood Research Station from 19-22 October 1987 at which 17 scientists from 7 countries participated. For detailed results, the reader is referred to the report which will appear shortly.
The main conclusions can be summarized as follows. The transfer of some radionuclides from plants to animals in the terrestrial environment after the Chernobyl accident has shown lower transfer factors than the values adopted so far which are based on studies in the laboratory and with fallout from weapon's testing.
This has been clearly established for the transfer of the Cesium isotopes but it has been found for I-131, also considerable attention has been given to the transfer of radionuclides in game animals, e.g. deers. Much more

information is needed on the transfer of radionuclides in young growing animals and in species such as sheep and goats. As was shown in the past, more study is necessary on the influence of the changing animal husbandry practices on the transfer of radionuclides to animal products.
c) Working group "Radioecology of Major Rivers".
The main activity of the group was devoted to the collect of informations about the radiological impact of the Chernobyl accident on the freshwater environment.
Several meetings between radioecologists from various countries provided a lot of good information. In order to produce an overview of the impact, a data base was constituted as well as an appropriate computer programme.
d) The Chernobyl accident has given to radioecology a new impulse and more than ever a real and effective collaboration between the European Research Centres is needed.
That explains why the IUR has in 1987 :
- brought to light the report on the actual and futur objectives in radioecology
- creates a new working group on radioecological assessment of radionuclides releases in the environment
- prepared a proposal within the field of the Erasmus Project in order to harmonize the educational formation in radioecology in Europe.

IV. Objectives for the next reporting period:

- Co-organisation IV Symposium International de Radioecologie Cadarache (Mars 88)
- Collaboration with the IAEA
  a) IUR has been invited by the IAEA to send a representative to the Advisory Group Meeting to consider the Effects of Ionizing Radiation on Terrestrial and Freshwater Aquatic Ecosystems (18-22 January,1988,Vienna).

A working document has been prepared by two consultants (Dr. G.Blaylock and Dr.W.Whicker, respectively from ORNL and University of Colorado, who are IUR's Members) as a basis for the discussion of the Advisory Group. It was prepared with the following objectives :
- to summarize the experimentally determined radiation doses which produce observable individual-,population- or ecosystem-level effects in freshwater and terrestrial biota;
- to estimate the possible radiation doses received by freshwater and terrestrial biota under current radiation protection standards;
- to determine whether the ICRP-26 statement concerning the protection of non-human organisms and populations is essentially correct.

A clear resolution of this question could be meaning ful with respect to the issue of whether or not radiation protection standards for aquatic and terrestrial biota are warranted.

b) Participation to a CRP (Radioecological Assessment Committee) IAEA (May 88) (see WG activities)

c) IUR will take part as an "Observer" in the IAEA International Conference on Radiation Protection in Nuclear Energy, which will be held the week following IRPA-7 Congress (Sydney, 10-17th April, 1988) of which the IUR's President is a participant.

- On official invitation from the Ministry of Foreign Affairs of the People Republic of China and from JINAN University (Guangzhou), IUR's Representatives will spend about three weeks in this country with the following objectives:
  - to improve the international cooperation in the field of radioecology;
  - to gain an overview of the Chinese Nuclear Industry;
  - to participate to Seminars on actual and future objectives in radioecology, specially after the Chernobyl accident.
- In the framework of the SCOPE-ENUWAR activities, IUR has been officially invited to send a representative to the forthessning ENUWAR Moscow Workshop, March 21st-26th 1988; a visit to Kiev and Chernobyl is also planned. One of the major objectives of this meeting is to re-appraise the consequences of a nuclear war on the non-human living organisms from the ionizing radiations.

Working Groups activities

a) "Soil-to-Plant Transfer Factors" Working Group will continue its activities, in particular the development of a statistical model which allows the integration of all data. The model should have a general applicability so that it can be used if no particular soil or climatic data are available, but it must supply refined values in case such data are available indeed.

b) "Plant-Animal" Working Group will issue the report of the meeting held at Merlewood (Grange-over-Sands,UK) 19-21 october 1987; this report will be circulated and will serve as a basis for further discussions in order to define the priorities in this field.

c) "Radioecology of Major Rivers". A meeting of the working Group is foreseen on the 16th March,1988, in Cadarache to collect the informations from each participant, to analyse the data and to prepare a synthesis on the radioecological consequences of the Chernobyl accident on the aquatic ecosystems.

d) The "Environmental Assessment Modelling Group" will continue to play a role in the BIOMOVS (Biospheric Model Validation Study) activities, the sixth coordination Group Meeting is planned in Budapest 25-30 April, 1988.

e) "Radioecological Assessment Committee" will participate to the first Research Coordination Meeting of the Coordinated Research Programme (CRP) on validation of models for the transfer of radionuclides in terrestrial, urban and aquatic environments, which will be held in Vienna (2-6 May,1988).

f) A new working Group on the "Marine Radioecology" will be launched and will work in close coordination with the CREST

programme (NEA/OCDE) and the MARINA (CEC), a meeting in June in Riso is foreseen.

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

VI. Publications

- Papers presented at the Vth Workshop of the IUR Working Group Soil-to-Plant Transfer Factors (Egham,UK, 14-16 April 1987)

Stoutjesdijk, J.F., van Ginkel, J.H., Pennders, R.M.J.:
Determination of soil-plant transfer factors and measures to influence the uptake of radionuclides.

Eriksson, A. and Rosen, K.:
Observations on the transfer of Cs-137 from soils to barley crops in Sweden after the Chernobyl fallout.

Cawse, P.A. and Baker, S.J.:
Atmospheric Deposition of Radionuclides and Transfer to Vegetation in Great Britain.

Juznic, K.:
On the Transfer of Radiostrontium from soil to plants.

Kirchmann, R. and Fagniart, E.:
Transfer Factor values observed in experimental field conditions and from Chernobyl fallout.

Mittelstaedt, W.:
Translocation of Cs-137 and 90-Sr in two German soils.

Schüttelkopf, H.:
Soil-Plant Transfert Experiments from '86 (and the following years) performed in the KFK.

Steffens, W.:
Transfer of Cs-137 and Sr-90 from soil into edible plant parts after simulation different types of deposition.

Desmet, G.:
Theoretical considerations about transfer factors with Tc as a model.

Information Bulletin of IUR n°7 (March 1987)
Specific needs of Developing Countries in the field of Radioecology - Report IUR distributed during the United Nations Conference PUNE, Geneva 23 March -10 April,1987.
Report on Chernobyl Poster Session presented during the Seminar CEC/JEN, Madrid, (15-19 September 1986) August 1987

III C

NICHTSTOCHASTISCHE WIRKUNGEN IONISIERENDER STRAHLEN

NON-STOCHASTIC EFFECTS OF IONIZING RADIATION

EFFETS NON-STOCHASTIQUES DES RAYONNEMENTS IONISANTS

RADIATION PROTECTION PROGRAMME

Progress Report

1967

Contractor: Contract no.: BI6-C-187-b

Univ. Catholique Louvain-la-Neuve
Halles Universitaires
Place de l'Université 1
B - 1348 Louvain-la-Neuve

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

Prof. H. Fazin
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 are 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.

II. Objectives for the reporting period:

The reporting period was devoted to:

i) completion of the study on rat B lymphocytes after a single exposure to a sublethal dose of irradiation administered at a high dose rate;
ii) restoration of B lymphocyte cell populations in irradiated rats after intravenous injection of B lymphocytes from different origin.

Moreover, we studied the long-term effects of prenatal or early postnatal irradiation of the immune system in rats. Finally, we contributed to a program for developing rat monoclonal antibodies against human leukocytes.

III. Progress achieved:

3.1. The effects of lymphocyte transplantation on the restoration of the B lymphoid system after a 5 Gy whole body irradiation, in the rat

In our last report, we described the system we are using, with two congenic rat strains which can be distinguish by their kappa light chains. In a new series of experiments, we have studied the production of serum IgM in LOU/C.IgK-1a rats given LOU/C.IgK-1b spleen cells. As an example, Figure 1 shows the IgM production by the transferred B lymphoid cells as well as by the host itself. Quite clearly, the transferred B lymphocytes, at least up to 60 days after the restoration, are the only one which synthetize the serum IgM.

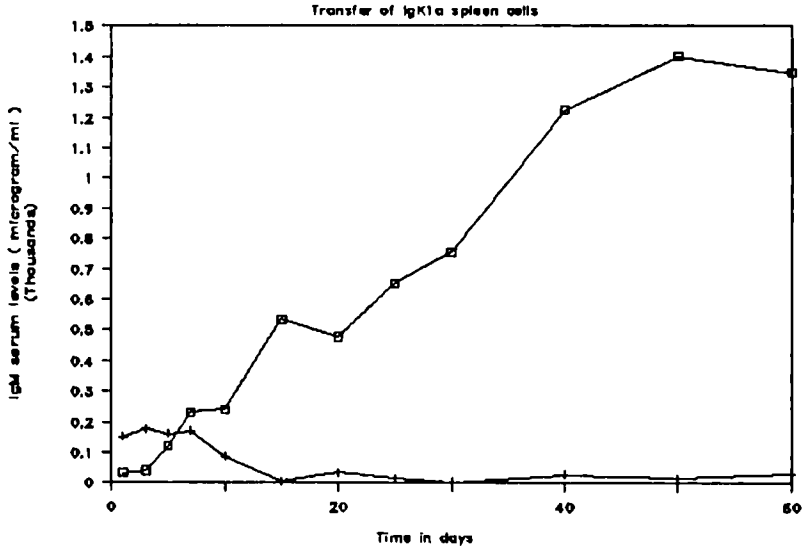


Figure 1: IgM serum levels in micrograms per milliliter of 5 Gy whole body irradiated LOU/C.IgK-1a rats given 150 x 10^6 spleen from LOU/C.IgK-1b, in function of the time after irradiation and restoration.

3.2. Lack of long-term effects of prenatal or early postnatal irradiation on the immune system in rats

In collaboration with F. Vander Plaetse and R. Hooghe (Pathology Section, Biology Dept, Study Center for Nuclear Energy, B-2400 Mol, Belgium), we have looked for long-term sequelae in the immune system of rat that had been irradiated on a single occasion (0-2 Gy, whole body irradiation, X-rays) during prenatal or early postnatal life. At the age of 2 months, the histology of the spleen was normal, and so were the amounts and distribution of B and T lymphocytes. The serum immunoglobulin levels were not altered, even when the different isotypes were considred. 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. Thus no long-term immunodeficiency could be documented in rats that had received up to 2 Gy before or early after birth.

3.3. Rat monoclonal antibodies against human leukocytes

In collaboration with the Immunohaematology Unit of the Faculty of Medicine of Louvain (Prof. G. Sokal), we are trying to develop rat monoclonal antibodies able to block a non compatible graft rejection.

Two hybridomas produced by fusion of the non-secreting rat myeloma line IR983F and splenocytes of a LOU rat immunized against phytohemagglutinin (PHA)-activated human blood lymphocytes, secrete IgG2b monoclonal antibodies (MAbs) called LO-Tact-1 and LO-Tact-2 whose reactivity is restricted to T lymphocytes activated by PHA, Concanavalin A or mixed lymphocyte reaction (MLR). Normal lymphoid cells of blood, tonsils or thymus were negative, as were malignant lymphoid or myeloid cells of B-CLL, T-ALL, AML, CALL and B-lymphomas or equivalent cell lines. Both MAbs recognize the same 65 kDa molecule (immunoprecipitation, followed by SDS-PAGE) and inhibit competitively the binding of ^{125}I-labelled interleukin-2 (IL-2) to PHA-activated T cells with KI estimated at 1.2×10^{9} M^{-1} (LO-Tact-2) and 8.4×10^{8} M^{-1} (LO-Tact-1). Both MAbs inhibit strongly MLR-induced proliferation, even if added 3 days after initiation of MLR. LO-Tact-1 mediates cell killing by Killer cells (ADCC). LO-Tact-1, a rat MAb of the K-1a allotype, has been produced in K-1b rats and purified, free from polyclonal antibodies, by immunoaffinity chromatography on mouse anti-rat IgK-1a MAbs. Hence, LO-Tact-1 and LO-Tact-2 are directed against the human IL2 receptor. From the in vitro assays, it can be inferred that when injected into allograft recipients rejecting their grafts, they not only will stop the proliferation of alloreactive T cells by blocking the IL2-receptor, but they also might specifically deplete the recipient from alloreactive T clones.

IV. Objectives for the next reporting period:

During the next vear of the contract, we will carry on the present experiments in order to understand the ontogeny of the B lymphocytes and the possibility to restore the various B cell compartments. We also will carry on experiments concerning the possibility to block graft rejection.

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

Dr Robert Hooghe, CEN, Department of Radiobiology, Mol, Belgium.

VI. Publications:

Publications not mentioned in the report of 1986

Cerny A., Huegin A.W., Sutter S., Bazin H., Hentgartner H. & Zinkernagel R.M. Immunity to lymphocytic choriomeningitis virus in B cell-depleted mice: evidence for B cell and antibody independent protection by memory T cells. Eur. J. Immunol. 1986, 16: 913-917.

Pear W.S., Ingvarsson S., Steffen D., Munke M., Francke U., Bazin H., Klein G. & Sumegi J. Multiple chromosomal rearrangements in a spontaneously arising t(6;7) rat immunocytoma juxtapose c-myc and immunoglobulin heavy chain sequences. Proc. Natl. Acad. Sci, 1986, 83: 7376-7380.

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Publications of 1987

Pear W.S., Wahlstrom G., Nelson S.F., Ingvarsson S., Bazin H., Klein G. & Sumegi J. C-myc activation in spontaneous rat immunocytomas containing a 6:7 chromosomal translocation. Current Topics in Microbiology and Immunology 1986, 132: 169-174.

Hutschemackers J., Bazin H. & Verhoyen M. Utilisation d'anticorps monoclonaux dirigés contre le potato virus X. 34ème Symposium International Phytopharmacie et Phytiatrie, 1987.

Uher F., Puskas E., Gergely J. & Bazin H. An IgM-producing immunocytoma induces large numbers of splenic T lymphocytes with fcμ receptors. Immunology 1987, 61: 327-332.

Hutschemackers J., Verhoyen M. & Bazin H. Production d'anticorps monoclonaux de rat spécifiques à Erwinia amylovora. Bulleting OEPP/EPPO, 1987, 17: 211-218.

Bazin H. Rat-rat hybridoma formation and rat monoclonal antibodies. Methods of Hybridoma Formation 1987, ed. A.H. Bartal & Y. Hirshaut, Humana Press, Clifton, New Jersey.

Schumcker D.L., Glibert R., Hradek G.T., Jones A.L. & Bazin H. Effect of aging on the hepatobioliary transport of dimeric immunoglobulin A in the male Fisher rat. Gastroenterology (in press).

Bazin H., LOUVAIN rat immunocytomas. Adv. Cancer Res. 1987, 50: 279-310 (in press).

Communications

Accurate determination of immunoglobulin isotype from hybridoma culture supernatants by Elisa. Contact Group Monoclonal Antibodies. Meeting of November 29, 1985. Limburg Universitair Centrum, Diepenbeek. In collaboration with P. Manouvriez & J. Smyej.

Cytotoxicité anticorps dépendante par les cellules K: médiation par des anticorps monoclonaux de rat d'isotypes différents. Association pour la Recherche sur le Cancer, I.R.S.C., 31 janvier - 1er février 1986. Book of abstracts E-106. In collaboration with D. Chassoux & G. Linares.

B lymphocyte generation after a single whole body irradiation dose of 5 Gy. 6th International Congress of Immunology, Toronto, Canada, 6-11 July 1986. Book of abstracts 1.11.7. In collaboration with B. Platteau.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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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
Mr. N. J. North

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:

(a) To investigate the "anomalous MD-50 data" for 1 mm, Sr-90 and Tm-170, sources.

(b) To commence parallel MD-50 studies with Curium-244 alpha emitting sources (held up in 1986 due to lack of source from a collaborating laboratory).

(c) To extend the DNA LI studies and the histological study of post irradiation re-epithelisation begun in the first 6 month period of the contract. This should clarify the relative roles of cells, both inside and outside the radiation field, in the process of epithelisation.

III. Progress achieved:

Methodology

A. Moist Desquamation Experiments

The 1986 annual report gave the essential details of the experimental procedures used to assess the acute moist desquamation reaction of mouse skin to a variety of beta sources. These sources include strontium-90, thulium-170 and promethium-147. The source sizes varied from 0.1 mm - 15 mm diameter.

Results

Work this year has on the whole confirmed the threshold and 50% effect doses (MD-50s) given in the 1986 report and shown the same area : dose response relationships. The MD-50 doses varied from 28 Gy for the large (64 mm^2) thulium-170 to 800 Gy for the 20 mm^2 promethium-147. However, for this latter source, only some 75% of mice exhibited moist desquamation, despite using doses up to, and including, 1500 Gy. For the smallest promethium-147 source (3.1 mm^2), no moist desquamation was seen at any level over the same dose range (50 - 1500 Gy). The threshold doses, when fitted by eye, varied from 10 - 12 Gy for the 64 mm^2 thulium-170 to 140 Gy for the 0.8 mm^2 strontium-90 and still appeared to show an area effect. However, when the thresholds were calculated using the logit fit analysis, the skew effect pushed many of the threshold values to the left, particularly for the sources where a high dose was required before a 100% maximum MD was observed. Using those values, any area effect was lost. Analytical work now continues to establish more accurate, meaningful criteria.

In 1987, we extended our experiments to include two smaller thulium-170 sources of 0.1 and 0.5 mm in diameter and as predicted the threshold and MD-50 doses are commensurately larger; for example, the threshold dose for the 0.1 mm source was approximately 150 Gy, whilst the MD-50 value for the 0.5 mm source was 550 Gy. As seen with the small area promethium sources, the highest doses did not produce a 100% MD effect. However, the extremely low dose rates of both sources prevented further investigation into higher doses.

One of the objectives of this reporting period was to investigate the "anomalously high" MD-50 value for the 0.8 mm^2 strontium-90 (709 Gy) when compared with an MD-50 values of 228 Gy for the 0.8 mm^2 thulium-170 source, a less energetic beta-emitter. The detailed dosimetry of these

two sources should shed some light on this discrepancy, but it is proving difficult and we still await data from a collaborating laboratory. Meanwhile we have begun a series of histological investigations of the lesions produced by these two small sources. The histopathology is yet to be completed, but initial studies appear to show that the small strontium-90 source response was possibly undervalued.

The second objective of this 1987 reporting period was to have begun some parallel acute moist desquamation studies with curium-244 alpha sources. The fabrication of this special source was only completed, after a serious delay, by the UK Atomic Energy Authority in the final month of the year and we have as yet no biological data to report.

The third objective was to study the DNA labelling indices of the basal cells of the mouse skin to determine the relative roles played by follicle and field-edge epithelial cells in the regenerative skin response. These indices will be compared with the use of vincristine sulphate studies of metaphase arrest to measure basal cell birth rate. The labelling and birth rate studies of a 5 mm lesion using the three sources of different energies - promethium-147, thulium-170 and strontium-90 have been begun. Initially a time course study was carried out using vincristine sulphate alone, the animals being killed at 10 post-injection times between 20 and 210 minutes. This established 90 minutes post-injection as the possible optimum time for metaphase arrest. Further studies have now been done involving the above three sources; analysis of these data are not yet completed.

IV. Objectives for the next reporting period:

(i) 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.

(ii) 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 mouse skin.

(iii) 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.

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
Mr. N. J. North

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 irradiation and so test the ICRP assumption that for a given average tissue dose inhomogenous 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:
(a) Complete the exposure programme for the thulium-170 sources. Continue monitoring the mice for tumours/morbidity.
(b) Commence the skin cancer induction project using one large ($\geqslant 500$ mm^2) and one smaller ($\leqslant 50$ mm^2) curium-244 alpha sources.
(c) Monitor the four strains of mice for their cancer proneness and develop the histological procedures to quantitate the variations in possible target cells such as melanocytes, dedifferentiated keratinocytes and hair follicle basal cells.

III. Progress achieved:

Methodology

A. Uniform, Thulium Dose Rate Experiments

Male SAS/4 albino mice 11-12 weeks old were used in all experiments. Prior to irradiation an area of dorso-lateral skin was chemically depilated. This has the effect of triggering resting (telogen) follicles into the synchronous active (anagen) cycle. This ensured that the epidermis and dermis were of a known thickness and that hair follicles were at a known depth in the skin. The mice were irradiated immediately after depilation while the follicles were still in telogen. Mice showing hair regrowth before day 9 after depilation/irradiation were excluded from the experiment since such early regrowth implied that the follicles had been in anagen and not telogen at the time of irradiation.

The thulium-170 sources were described in a previous progress report. The dosimetry of all sources was carried out using an extrapolation ionisation chamber with interchangeable collector electrodes, the smallest of which has an area 1.1 mm^2. All depth doses, spatial dose distribution and mean doses over the irradiated areas were expressed at 16 μm into the skin, this being the tissue equivalent of the chamber window. The beta doses at the bottom of the basal layer of the dermis are approximately 50% of the skin surface dose.

Of the three thulium sources used, two produced beta radiation at a high dose rate (10 Gy min^{-1}), whilst the third was at a lower dose rate (1 Gy min^{-1}). Sixty SAS/4 mice received 100 Gy from the high dose rate source, whilst a further sixty mice received 100 Gy from the low dose rate source.

The details of the mode of exposure have been described in the previous report, and following irradiation the mice were examined daily for six weeks during the acute skin reaction period and then checked daily for the appearance of skin tumours and any signs of morbidity. Post mortem examinations will be carried out on all animals and tumours will be histopathologically examined and classified.

B. Skin Cancer Induction Following Curium-244 Alpha Irradiation

These experiments have not been performed due to the lack of alpha sources but will commence as soon as possible in 1988.

Results

(a) To date, none of our 152 control animals have produced a tumour

within the experimental area. [These are animals which have undergone the anaesthesia/depilation procedure, but were not exposed to a radiation source].

450 animals were exposed to a uniform thulium-170 source at doses ranging from 2 - 300 Gy. These are life-time experiments, and some 10 - 15% of the animals still survive at all dose levels. Thus, all results are preliminary and will be subject to a final statistical analysis. Nonetheless, tumours have developed in all dose groups although the 'tumour' status has not been verified pathologically in all cases. Initial findings show a dose relationship as indicated by earlier work carried out in this laboratory. The cumulative tumour incidences at the 110 week post-irradiation point, when corrected according to the life table analysis, for the 2, 5, 10, 20 and 300 Gy groups are 5.4%, 7.1%, 4.6%, 27.2% and 48.7% respectively. These results confirm work done earlier: there is a dose-response relationship with respect to tumour incidence; also, as the radiation dose increases, the latent period for tumour appearance decreases. Initial pathological studies also indicate that the early tumours appearing 30 - 70 weeks post-irradiation have proved more likely to be of epidermal origin and thus are true 'skin' tumours, rather than of dermal origin. The actual nature of the majority of the dermal tumours has proved to be a matter for pathological discussion and calls into question the target cells within this tissue.

With respect to the non-uniform 8-array thulium-170 source, two groups of approximately 100 animals in each, were irradiated at 2 and 10 Gy. The cumulative tumour incidence at 110 weeks post-irradiation are 2.7% and 6.4% respectively, subject to the above contraints. The non-uniform 32-array source was of extremely low dose rate and only one group of 99 animals was irradiated at 2 Gy. Contrary to previous findings, the cumulative tumour incidence for this group was found to be higher than that for the equivalent uniform group at the same 110 weeks post-irradiation point: 11.81% compared with 5.36%. This high cumulative value is the result of five early-appearing tumours, the earliest of which has been classified pathologically as a basal cell carcinoma, a tumour not previously seen in our experiments with this strain of mice.

(b) The study into strain differences with respect to tumour induction is still relatively early due to the long latent period for skin tumours. However, initial findings are interesting. Two albino strains have been

looked at, SAS/4 and CD1, and these have been irradiated using the uniform thulium-170 source over a dose range of 12.5 - 100 Gy. The SAS/4 groups show a direct comparison to previous results, with respect to probable tumour incidence. However, too few animals have died to give meaningful data. The CD1s however show a marked dose response with a possible accelerated latent period. At the present (approximately 50 weeks post-irradiation) point, the four dose groups of 12.5, 25, 50 and 100 Gy have an estimated cumulative tumour incidence of 0%, 3.5%, 9.1% and 17.2%. These results are not confirmed histopathologically nor do they take into account any tumours present on animals still surviving. As yet, no tumours have occurred in the control CD1 animals.

By contrast, the brown CBA/ca animals have produced no tumours to date, whilst in the black C57BL/6 strain, the two highest dose groups of 100 and 50 Gy have cumulative tumour incidences of 3.8% and 1.9% respectively.

(c) With respect to the dose-rate experiments, no skin tumours were expected and none were recorded within the reporting period.

IV. Objectives for the next reporting period:

(a) 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.

(b) Continue to monitor the four strains of mice for the cancer proneness.

(c) Complete the exposure programme for the different dose-rate thulium-170 sources. Continue monitoring the mice for tumours/morbidity.

(d) Commence the skin cancer induction project using curium-244 alpha sources.

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.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no.: BI6-C-058-F

Commissariat à l'Energie
Atomique, CEA
B.P. n° 510
rue de la Fédération, 39
F - 75752 Paris Cédex

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 irradiation. Experimental study in the pig.

Head(s) of project:

Dr F. DABURON

Scientific staff:

Dr D. HOFFSCHIR, Dr J.L. LEFAIX, M. MARTIN, J. REMY.

I. Objectives of the project:

Diagnosis, prognosis and treatment of acute localized irradiated in pigs and rabbits. Dosimetric assays by non invasive biophysical methods to evaluate the size and intensity of the radiolesions as an aid for operating. Pathogenic studies of irradiated tissues by histological, histochemical, immunological and cellular culture methods.

II. Objectives for the reporting period:

- Visualization and signal intensity analysis on NMR tomograms, of the muscular tissue modifications after irradiation in rabbits : oedema, ischemia and necrosis.

- Evolution of 201 Tl uptake by irradiated muscle in pigs according to the chronology of the development of the radiolesions.

- Physiological and biochemical analysis of fibroblasts in culture, either normal pr extracted from radiation induced fibrosis.

- Molecular biology studies of the synthesis abilities of fibroblasts (collagens).

III. Progress achieved.

Methodology

- Acute localized irradiation were performed with a 192 Ir collimated source.
  - on the left part of the back (m. iliospinalis) of rabbits at doses ranging from 10 to 40 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 four times higher.

- Rabbits (4 control and 32 irradiated) were observed between day 2 and day 22 by NMR tomography.

- Pigs (3 at 30 Gy and 3 at 60 Gy) were followed from day 1 to day 45 by 201 Tl gamma scintigraphy.

- Fibrosis cells were extracted from 5 to 20 months after irradiation, seeded and subcultured every 7 days. Control cells were pig dermal fibroblasts.

Results

NMR tomography - Differences in the signal intensity (weighted by T2) between irradiated and normal muscle were quantified on NMR tomograms according to two parameters, proton density and proton density heterogeneity.

A multivariate analysis was performed on these data : after 4 successive tomographies (day 2 to day 8) it was possible to differentiate control from irradiated animals, when no clinical lesions could be pointed out, either at skin nor at muscle level.

201 Tl scintigraphy - The irradiated area on the pig thigh showed an early and protracted high uptake which was correlated with early erythema. The uptake remained very low during the clinical latency period.

During the second erythema wave a high uptake ring appeared round the necrozed area at the center of the lesion where no uptake could be noted.

This succession of scintigraphic images made obvious the different stages of the evolution of the radiolesions : first vascular reaction, then cellular reaction around the necrozed area.

Increasing the dose increased the uptake intensity and volume and reduced the delay between the different phases.

Fibrosis - We observed previously that fibroblasts extracted from pigs with radiation-induced scars had a proliferation potential in primary culture ten times higher than normal dermal fibroblasts.

Two characters of these cells were studied in 1987 : the in vitro lifespan and the response to growth factors.

- Growth curves of control fibroblasts fitted to a classical HAYFLICK curve, senescence taking place between the 10th and the 15th subculture.

Fibrosis fibroblasts did not present any senescence process. At the present time the oldest cell lines are over the 50th subculture ; after the 30th subculture cells, caryotypes were becoming polyploid.

- Growth factors. Aged cell lines showed an important density of FGF-b receptors on the cell membrane. The response to EGF stimulation, measured by the increase of the cell number in confluent phase, was two times higher for fibrosis fibroblasts.

- Molecular biology studies were carried out on fibrosis and normal fibroblast using cDNA probes hybridizing with mRNA of various collagens (I, III types). At the present time probes are amplified and their specificity is satisfactory although they were prepared on man or mouse; the size of the pig hybridized mRNA were determined in Northern blot.

- Discussion

The results obtained by the two biophysical methods above mentioned can be added to the others previously tested in pigs (microwave thermography, X and NMR tomography) to answer the questions stated by acute localized irradiation diagnosis.

MNR imaging in rabbits, despite the difference in species and muscle morphology, gives the same results as in pigs : no lesions can be visualized below 40 Gy.

With 201 Tl imaging in pigs the chronology and the intensity of the inflammatory reaction, local and peripheral, can be followed without reference to clinical manifestations.

The reason why fibrosis fibroblasts are so greatly different from dermal fibroblasts will be investigated at the cellular level as far as growth factors are concerned. At the molecular level we would like to determine if the excess of extracellular matrix synthesis is due to modifications at transcriptional or gene activation level.

IV. Objectives for the next reporting period:

- Development of NMR studies in rabbits : tomography and 31P spectrometry.

- Development of 201 Tl scintigraphy in pigs in relation with electrophysiological studies.

- Growth factors involvements in post irradiation fibrosis.

- Molecular biology studies : measures of the cellular level (mRNA collagens) in fibroblasts from fibrosis, normal tissue or cell cultures.

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 Dr 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 CRETEIL (Prof. Robert).

- CEA Institut de Recherche et de Développement Industriel. Laboratoire d'Electronique et de Technologie de l'Informatique CEN-GRENOBLE (MM. Allemand et Jeandey).

VI. Publications:

1.
DABURON F., LEFAIX J.L., REMY J., FAYART G., TRICAUD Y.- Mesures thermographiques microondes apres irradiation localisée chez le porc. Méthodes d'acquisition et de traitement. (ITBM 1987, 8, 367-383.

2.
DABURON F., LEFAIX J.L., REMY J., HOFFSCHIR D., FAYART G.- Biophysical methods for the diagnosis of acute localized irradiation : experimental studies.
Com. E41-8V-8th Int. Congr. of Radiation Research. Edinburgh 19-24 July 1987.

REMY J., MARTIN M., WEGROWSKI J., DABURON F. - Fibrosis : late effect of local radiation damage - in vitro studies - Com. E22-22P-8th Int. Congr. of Radiation Research, Edinburgh 19-24 July 1987.

WEGROWSKI J., EL NABOUT R., LAFUMA C., MARTIN M., and DABURON F.- Collagen and fibronectin modifications in radiation-induced muscular fibrosis of the pig. in vivo and in vitro studies. The control of Tissue Damage 75th Anniversary Symposium. The Strangeways Research Laboratory, Cambridge, 6-8 April 1987, 39-42.

LEFAIX.J.L,DABURON.F,CRECHET.F,TRICAUD.Y.
Conséquences plasmatiques et thermographiques d'une irradiation aigue localisée.Analyse qualitative et quantitative chez le porc.
Rapport CEA R-5395,1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.

Contract No: B16-C-176-IRL.

Head(s) of project: Dr. Geoffrey Dean. MD,FRCP.,FRCPI. FFCM.FFCMI.

Scientific staff: Professor Michael Marmot, Professor of Community Medicine, University College,London. (Collaborating with study).

Scientific Advisers: Dr. Ebert and Dr. G.B.Gerber, European Economic Community, Brussels.
Dr. Remy Maximilien, Euratom, Fontenay-aux-Roses. France.

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 will be asked to provide a subsequent history of the patients, whether they had developed cataract and whether the cataract had been removed. A comparison will then be made between those who had radiotherapy and those who had alternative treatment. A previous small study suggests that those who had received radiotherapy had a greatly increased risk of cataract.(1)

II. Objectives for the reporting period:

To obtain details of the hospital records of 500-600 patients who had received radiotherapy for inflammatory eye conditions between 1950 and 1970 and details of therapy of a similar number of patients suffering from similar eye conditions who had received other treatment. To trace these patients through the National Health Service Central Register, Southport.

III. Progress achieved:

1. METHOD

Five hundred and fifty nine records were obtained and cards completed for them for patients who had had radiotherapy to the eyes.

Records were obtained from the following hospitals:

1. Central Middlesex	321
2. Newcastle General Infirmary and Royal Informary	199
3. Wolverhampton Royal Infirmary ...	7
4. St. Bartholomew's Hospital, London.	16
5. Bristol General Hospital, Bristol. ...	16
Total	559

To match these patients 580 "controls", i.e. patients who had received therapy to the eye other than radiotherapy, were obtained from St.Thomas' Hospital, London. The control patients had received local therapy such as cortisone eye drops or argyrol, a silver preparation.

For each patient a card was completed giving the basic data and a second card describing the exact treatment received by the patient. The cards were photocopied and the outer card giving the basic data about the patient was forwarded to the National Health Service (NHS) Central Register, Southport, for tracing.

2. RESULTS

Four hundred and nine patients were traced by the NHS, Southport, of whom 168 had received radiation therapy and 241 were controls. The remainder had died, emigrated or were not traceable because there was insufficient data on the original hospital records.

The National Health Service Central Register in Southport has the name and address of the local Family Practitioner Committee where the traced patients are presently registered but does not have the name and address of the patient's doctor. This is kept by the local Family Practitioner Committee. The NHS in Southport is therefore sending a letter to all the Family Practitioner Committees where patients are registered, enclosing for them a letter to send on to the doctor concerned with a stamped addressed pre-paid envelope and a simple form for reply.

In order to obtain the maximum response from the doctors, Professor Michael Marmot is collaborating in the study. He is Professor of Community Medicine at University College, London. The general practitioners are more likely to reply to this address than to a government agency. When the doctors do not reply to the first letter, a second and then, if necessary, a third letter will be sent, followed by a telephone enquiry if necessary.

3. DISCUSSION

The study is going well and has now reached the stage when we are writing to the Family Practitioner Committees and asking them to forward a request for details about the present health of the patients to the patients' general practitioners. With the letter to the patient's doctor will go a form for the doctor to complete which will provide the data required for analysis, in particular whether the patient has developed a cataract, whether they have seen an ophthalmologist about the cataract, who the ophthalmologist was and at which hospital, and also what information they have as to the characteristics of the cataract.

IV. Objectives for the next reporting period:

To obtain replies from the patients' doctors as to the patients' subsequent eye history after they had received treatment with radiotherapy or alternative therapy. In particular to ascertain how many developed cataract in the two groups in comparison with the general risk of developing cataract, or being operated on for cataract, in the general population, age and sex corrected.

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

None.

VI. Publications:

(1). Dean G, Alderson M, Maximilien R. Increased risk of cataract in patients receiving radiotherapy to the eye. British Journal of Radiology. Accepted for publication.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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. Modulation of IL-2 production in irradiated mice by thymosin α_1.

2. Mapping of antigenic epitopes that interact with MHC class II molecules and the T cell receptor.

III. Progress achieved:

Expression of the remarkably wide repertoire of effector immune functions is modulated by cellular and molecular interactions in a complex immunoregulatory network. Activation of effector cells is induced by macrophage-processed antigen and modulated by signals passed among different cell types that regulate the intensity and duration of the immune response. Interleukins play a major role in the amplification of immune responses. Thus, antigen-stimulated macrophages release interleukin 1 which in turn induces the production and release of interleukin 2 (IL-2) from T cells upon activation by antigen in association with MHC class II molecules. IL-2 promotes proliferation of activated T and B cells expressing IL-2 specific receptors.

Our previous studies had shown that unprimed helper T cells in mice are very radiosensitive and recover slowly, as some functional defects are still detectable 3 months after 200 rad and complete recovery is reached only 6 months after 400 rad. Recovery of helper T cell function from radiation damage is accelerated by injecting mice with synthetic thymosin α_1, a 28 aminoacid residues peptide identified in the bovine thymus extract Fraction 5 and very effective in the induction of T cell markers and functions.

Since irradiation is known to impair both T cell helper activity and IL-2 production, experiments were performed to determine whether the injection of thymosin α_1 in irradiated mice can also restore IL-2 production. Three month old BDF1 mice were total-body exposed to 100, 200, 300, or 400 rad of X rays and given a single injection of 10 ug thymosin α_1 1 hour, 4, 7, or 14 days after irradiation. Spleen cells were harvested 3 days after thymosin injection and assayed for IL-2 production upon in vitro stimulation by Concanavalin A and Phorbol Myristic Acetate. Results from this series of experiments show that IL-2 production is reduced by irradiation and may be restored to some extent by a single injection of thymosin α_1, the recovery being more pronounced when thymosin α_1 is given early after small radiation doses. In a subsequent series of experiments mice exposed to 100, 200, 300, or 400 rad were given 3 injections of 1, 10, or 100 ug thymosin α_1 1 hour, 1 and 2 days after irradiation. Spleen cells were harvested the day after the last thymosin injection and assayed for IL-2 production. Results from two experiments indicate that maximum recovery of IL-2 production is attained by 3 injections of 10-100 ug thymosin α_1 after 100-300 rad (100) or 400 rad (55-70). These preliminary findings are very promising as they clearly indicate that thymic hormones are powerful immunoregulatory molecules that may be successfully used for treatment of T cell immunodeficiencies in accidentally or therapeutically irradiated persons.

Our studies on the characterization of the T cell receptor have been extended to investigate functional aspects. Synthetic peptides of hen egg-white lysozyme (HEL) have been used to analyse an immunodominant epitope for T cells of the H-2^d haplotype that is contained in the HEL sequence 107-116 and is recognized in association with I-E^d molecules. The immunodominance of this epitope in HEL-primed H-2^d mice was demonstrated by the analysis of the T cell proliferative response induced by synthetic peptides covering the entire HEL sequence. All the T cell hybridomas analyzed recognize the HEL sequence 107-116 as defined by the use of N- and C-terminal truncated synthetic peptides. Conservative, or semi-conservative, substitutions at positions 113 (Asn to Lys), 114 (Arg to His) or 115 (Cys to Ala) abrogate the ability of peptide 105-120 to activate T cells. Substitutions at residues 113 and 115 affect T cell recognition but not the binding to I-E^d molecules whereas, as shown by binding data and competition experiments, an Arg to His substitution at position 114 profoundly impairs the capacity of the peptide to interact with I-E^d molecules. In agreement with these results, immunization of H-2^d mice with peptide (Lys^{113}) HEL 105-120 induces a T cell proliferative response specific only for the immunogen, whereas immunization with (His^{114}) HEL 105-120 fails to induce any detectable T cell activation. Thus, a strict correlation exists between binding of a peptide to Ia molecules and its immunogenicity, since a single semi-conservative substitution drastically reduces binding capacity and abolishes immunogenicity. This indicates that the MHC molecules are able to discriminate between very similar structures and may, in part, explain how the limited diversity of the class II molecues expressed by any given individual could select relevant epitopes from among the almost endless variety of protein peptide antigens.

IV. Objectives for the next reporting period:

1. Role of thymic hormones and interleukins in radiation damage and recovery.

2. Interactions of immunodominant epitopes with Ia molecules in T cell activation.

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

VI. Publications:

1. De Santis R., Palmieri G., Doria G., and Adorini L.
T cell receptor-homologous mRNA from a suppressor T cell clone directs the synthesis of antigen-specific suppressive products.
Eur. J. Immunol. 17: 575-578, 1987.

2. Doria G., Mancini C., Frasca D., and Adorini L.
Age restriction in antigen-specific immunosuppression.
J. Immunol. 139: 1419-1425, 1987.

3. Doria G., and Frasca D.
Thymic hormones as immunoregulatory molecules: an overview.
In: Tumor immunology and immunoregulation by thymic hormones, F. Dammacco ed., Masson, Milano, 1987, pp. 109-121.

4. Doria G.
Perche' perde colpi il network dell'anziano.
In: Corriere Medico, 61: 10, 1987.

5. Frasca D., Adorini L., and Doria G.
Enhanced frequency of mitogen-responsive T cell precursors in old mice injected with thymosin .
Eur. J. Immunol. 17: 727-730, 1987.

6. Doria G., Adorini L., and Frasca D.
Immunoregulation of antibody responses in aging mice.
In: Aging and the Immune response, E.A. Goidl, ed., M. Dekker, Inc., New York and Basel, 1987, pp.143-176.

7. Barcellini W., Meroni P.L., Frasca D., Sguotti C., Borghi M.O., Uberti-Foppa C., Buzzetti P., Lazzarin A., Doria G., Moroni M., and Zanussi C.
Effect of subcutaneous thymopentin treatment in drug addicts with persistent generalized lymphadenopathy.
Clin. Exp. Immunol. 67: 537-543, 1987.

8. Meroni P.L., Barcellini W., Frasca D., Sguotti C., Borghi M.O., De Bartolo G., Doria G., and Zanussi C.
In vivo immunopotentiating activity of thymopentin in aging humans: increase of IL-2 production.
Clin. Immunol. Immunopathol. 42: 151-159, 1987.

9. Frasca D., Adorini L., Landolfo S., and Doria G.
Pleiotropismo dell'attivita' immunoregolatoria di IFN-γ.
In: XV Convegno Naz. Gruppo di Cooperazione in Immunologia, Cortona, 1987. Abstract p. 84.

10. Adorini L., and Doria G.
Interazioni fra peptidi sintetici del lisozima, molecole MHC di classe II e recettore T.
In: XV Convegno Naz. Gruppo di Cooperazione in Immunologia, Cortona, 1987. Abstract p. 50.

11. Adorini L.
Il secondo recettore dei linfociti T.
In: Aggiornamento del Medico 11: 703, 1987.

12. Adorini L., Bove C., Darsley M., Appella E., and Doria G.
Mapping of antigen epitopes interacting with class II MHC products and with the antigen receptor of T lymphocytes.
In: Macromolecular Biorecognition. Principles amd Methods. I. Chaiken, E. Chiamone, A. Fontana, P. Neri, Eds. Humana Press, Clifton, N.J., 1987, pp. 279-290.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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,CBiol

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 enhance late or chronic radiation damage.

II. Objectives for the reporting period:

1. Completion and analysis of RBE and low dose/fration study of 61 MeVp-Be neutrons

2. The investigation of the effects of irradiation on blood flow in the cervical cord using C^{14}-iodoantipyrine and quantitative autoradiography

3. The presymptomatic leakage of C^{14}-aminoisobutyric acid will be analysed using quantitative autoradiography

4. The observations made in a preliminary study of the reduction in radiation myelopathy by the use of vasoactive drugs will be extended and quantified.

III. Progress achieved:

1. RBE at low doses/fraction of 62p Be neutron and 16d Be neutrons.

The topping-up technique was used to measure RBE for the induction of radiation myleopathy in the cervical cord at doses/fraction within the therapy range. Six doses in 25 days of 0.6 Gy 250 kV Xrays, 1.5, 1.0 or 0.6 Gy 16d-Be neutron Hammersmith beam or 1.37 Gy 62p-Be neutron Clatterbridge beam were compared by topping up the fractionation, with top-up doses of Xrays. The irradiation boxes had 6mm Perspex walls to ensure full build-up from X rays and 16d-Be neutrons. For the 62p-Be neutrons 1 mm Polythene was added to the front wall to give full build up. For the 62p-Be neutrons the irradiations were at 2 cm deep in a Perspex phantom using the normal flattening filters of the clinical beam. The data combined with the normal fractionation data suggests a limiting value of RBE (relative to 250kV Xrays) of 8 - 9 for 16d-Be neutron and ~ 8 for 62p-Be neutrons. At a dose equivalent to 2 Gy $_{\gamma}$ rays the RBE's for 16d-Be and 62p-Be neutrons are 4.7 and 4.0 respectively giving a neutron dose ratio (NDR) of 62p-Be neutron/16d-Be neutron of 1.17. Observations using the jejunal crypt assay have shown that the RBE for the 62p-Be neutron beam falls as the beam penetrates the phantom by 11% and the NDR increases by 11% at 12 cm deep in the Perspex phantom.*

* Hornsey S, Myers R, Parnell C.J., Bonnett D.E., Blake S.W. and Bewley D.K, 1988. RBE and change in RBE with depth of the Clatterbridge neutron therapy beam.

British Journal of Radiobiology in press.

2. The investigation of the effects of irradiation on blood flow in the cervical cord using C^{14} iodoantiopyrine and quantitive autoradiography.

C^{14}-iodoantipyrine (IAP) was innoculated into the femoral vein of unirradiated rats and of presymptomatic rats following 30Gy of Xrays to the cervical cord. The animals were killed 30 seconds after IAP and the cords frozen and sectioned. Autoradiographs were scanned and counted to measure the transfer of IAP into

parachyma which is directly related to blood flow. In the unirradiated cord the variation in blood flow between regions of high vascularity and low vascularity was greater than a factor of 6. In the irradiated cord from 18 weeks pi. onwards there were regional reductions in blood flow in dorsal white and grey matter. These occurred in the absence of major histopathological changes but were associated with minor vascular lesions e.g. slight oedema and extravascular of erythrocytes.

3. Presymptomatic leakage of C14-aminoisobuteric acid (AIB) across the blood brain barrier

Presymptomatic leakage in rats following a dose of 30Gy Xrays to the cervical cord was measured. This dose causes ataxia in all animals between 20 and 24 weeks postirradiation. The leakage was measured by scintillation counting of tissue taken at various times following innoculation of C^{14}-AIB into the femoral vein. The activity was compared from sections of cervical cord, thoracic cord and the trigeminal ganglion. AIB normally crosses the blood brain barrier at very low rate and is virtually undetectable in the parenchyma of normal barriered CNS. If the permeability of the blood brain barrier increases the transfer rate of the molecule increases and it is rapidly taken up from the cerebrospinal fluid into the parachymal cells and sequestered, providing therefore, an excellent marker of changes in permeability. The blood brain barrier does not operate in the trigeminal ganglion and AIB passes freely into the parenclyme there. Changes in the level of permeability elsewhere in the CNS is therefore compared with that in the trigeminal ganglion.

Vascular permeability in presymptomatic animals was found to be increased from 18 weeks post irradiation onwards by factors of between 3 and 6 over control animals while in animals exhibiting ataxia these factors were increased further to between 8 and 13.

4. Effect of vasoactive drugs on the developing radiation myleopathy

Two vasoctive drugs have been used; dipyridymole (Persantin) a smooth muscle relaxant which increases blood flow and verapamyl

(Cordilux) a Ca^{+} channel blocker. In addition the drug desferrioxamine (Desferal) an iron chelating agent in combination with a reduced iron diet was used to reduce reperfusion injury. The cervical cords of 3 month old male rats were irradiated with 27Gy, 25 Gy or 23 Gy 250kV Xrays. Animals to be treated with desferrioxamine were put on a low iron diet at 84 days post irradiation. All drug treatment was started at 119 days post irradiation. Drugs were given three times weekly.

After 27 Gy all animals receiving no drug treatment developed ataxia within 143-193 days post irradiation. Ataxia developed later in all drug treated groups. In animals treated with dipyridymole or desferrioxamine 30 - 40% of animals had not developed ataxia at 240 days post irradiation. If the drug treatment was discontinued at 240 days post irradiation ataxia developed within three weeks. Verepmyl also delayed the development of ataxia and reduced the proportion of animals which had developed ataxia in the first 8 months post irradiated by 20%. After lower doses of irradiation the development of ataxia is delayed. Observations on these animals is continuing. All three drugs used delayed and reduced the development of ataxia in a dose modifying manner.

IV. Objectives for the next 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 C^{14}-AIB
3. Quantitative assessment of the variations in regional blood flow in irradiated cervical cord when new computing hardware and software become available

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

VI. Short Publications:

Hornsey S, Myers R and Tozer G.M. 1987
Vascular changes following irradiation of the Spinal Cord
6th Annual Meeting European Society for Therapeutic Radiobiology and Oncology, Lisbon. Abstracts p.233

Hornsey S, and Myers R. 1987
RBE for 62MeVp-Be neutrons and 16 MeVd-Be neutrons measured at doses within the therapy range
Proceedings 8th International Congress Radiat.Research, Edinburgh p.247

Myers R, Tozer GM and Hornsey S. 1987
Microvascular changes in irradiated Rat Spinal Cord
Proc.8th International Congress Radiat.Research, Edinburgh p.266

Hornsey S and Myers R. 1987
Relative Biological Effectiveness for the Spinal Cord at Clinical Values of dose/fraction
Proc. EORTC Heavy Particle Therapy Group <british Journal of Radiobiology 60. p 315

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Prof. Dr. T.M. Fliedner
Abt. für Klin. Physik & Arbeitsmed.
Universität Ulm
Oberer Eselsberg M 24
D - 7900 Ulm/Donau

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 inhomogeneus 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 irradiation, 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:

With respect to the specific objectives of this project it was of major importance to get as comprehensive information as possible about the distribution of the hemopoietic tissue, its activity in different parts of the skeleton and its immediate response to ionizing radiation. Therefore, the concentration of the early erythroid progenitor cells (BFU-E) in different bones and bone marrow sites was determined in normal dogs, and their radiosensitivity was assessed by in vitro irradiation. The changes in the BFU-E compartment were studied in dogs which received a total body irradiation with an inhomogeneous dose distribution.

III. Progress achieved:

1. Methodology

Bone marrow samples for the assessment of the BFU-E concentration were obtained from different bones. In some cases the bone marrow was scraped out from a resected piece of the humerus. Erythrocyte-poor cell suspensions were obtained from the bone marrow samples by either sedimentation in the presence of dextran (1.5%) or by density gradient separation over Lymphoprep (density 1.077 g/ml). The cells were cultured in methylcellulose either in the presence of 20% fetal calf serum (FCS) or a mixture of 20% FCS and 10% serum from lethally irradiated dogs (TBI-DS) and stimulated by erythropoietin, 2-2.5 IU/ml. The irradiations of the bone marrow cell suspensions were performed with 280 kVp X-rays (HVL = 2 mm Cu; dose rate 0.70 Gy/min). The total body irradiations were performed with 300 kVp X-rays (HVL = 4 mm Cu; dose rate 6.5 cGy/min on the middle axis of the dogs). An inhomogeneous dose distribution in the animals was obtained by an unilateral exposure, i.e. they were irradiated with their left side directed to the radiation source.

2. Results

a) The different separation procedures applied to the bone marrow samples obviously were of little influence if any on the growth of BFU-E in the cultures. However, colony formation could be increased by a factor of 1.5 if instead of 20% FCS a mixture of 20% FCS and 10% of TBI-DS was added to the cultures. There was the tendency for the humeral marrow to have higher concentrations of BFU-E/10^{-5} b.m. cells than the bone marrow of the iliac crest and the sternum. But in any case the BFU-E values were significantly lower than the GM-CFC numbers, i.e. between 43 and 118/10^{-5} b.m. cells in comparison to between 180 and 350/10 b.m. cells for the latter.

b) For the determination of the radiosensitivity of BFU-E suspensions of mononucleated b.m. cells were irradiated in vitro with increasing radiation doses. Thereafter, the cells were cultured in methylcellulose. The survival fractions obtained could be fitted by a simple exponential curve $N/No = e^{-D/Do}$ resulting in a Do = 0.16 Gy. Thus, the BFU-E are extremely radiosensitive when compared to the granulocyte/macrophage progenitor cell (GM-CFC), for which under similar conditions a Do = 0.55 Gy was obtained.

c) The inhomogeneous irradiation was characterized by the following radiation doses: entrance dose 3.8 Gy, midline dose in the body approx. 2.6 Gy and exit dose 0.9 Gy. The hematological effects of this exposure as observed in the changes of the blood leukocytes were rather similar to those found in dogs that had received a homogeneous total body irradiation (bilateral exposure) with a radiation dose of 2.4 Gy. In the unilaterally exposed animals the BFU-E concentration was determined in the bone marrow in the head of the humerus that was directed away from the source, i.e. was protected by the body and thus received less damage than the other bone marrow sites. On the first day after exposure the BFU-E had dropped to values between 1% and 8% of the initial concentration as predicted on the basis of their extremely low Do-value. However, in the interval between day 1 and day 14 after irradiation the BFU-E concentration showed a rapid increase to between 150% and 250% of the preirradiation value in contrast to the GM-CFC that showed some repopulation within the same period, but had reached only approximately 25% of the initial value. Thereafter the BFU-E remained in the normal range or slightly above up to 1 year after irradiation. The GM-CFC values as determined in the same bone marrow sites were found still subnormal at day 48 and 60 and had normalized at day 125.

3. Discussion

The concentration of BFU-E in different bone marrow sites is not uniform. However, there is obviously a close correlation between the high GM-CFC concentration and the high BFU-E values in the red marrow of the head of the humerus. In the other bones the concentration of both is lower. On the other hand the canine BFU-E has been found to be extremely radiosensitive when compared to the GM-CFC. It is interesting to notice that the survival curve is strictly exponential i.e. there is no indication of sublethal damage. However, it has to be questioned whether the survival of BFU-E can be improved by certain changes in the culture conditons. The results obtained from the BFU-E determinations in the dogs which received an inhomogeneous TBI indicate that the BFU-E and the GM-CFC may show rather different repopulation kinetics in identical bone marrow sites after certain irradiation conditions.

IV Objectives for the next reporting period.

The high radiosensitivity as determined for canine BFU-E needs further exploration. The BFU-E is a relative immature hemopoietic cell with low proliferative activity in vivo. Thus, it will be of interest to study whether the radiosensitivity will be influenced by recruitment and proliferation due to specific growth factors. The acute response and late changes in the BFU-E compartment will be studied in dogs, which received homogeneous TBI. Preliminary results indicate that progress will be made in characterizing canine pluripotent hemopoietic cells (GEMM-CFC).

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 European research laboratories "compare notes" on these irradiation studies.

VI. Publications:

1. Publications in scientific journals

Baltschukat, K., W. Nothdurft and T.M. Fliedner (1987)
Hematological effects in dogs after irradiation of the lower part of the body with a single myeloablative dose.
Radiother. Oncol. (submitted 1987)

Fliedner, T.M., T. Szepesi and K.H. Steinbach (1987)
Medizinische Versorgung der vom Reaktorunfall Tschernobyl unmittelbar betroffenen Personen.
In: Niklas, K., W. Börner, F. Holeczke und O. Messerschmidt (Hrsg.): Tschernobyl und die Folgen. Begutachtung von Strahlenschäden [Strahlenschutz in Forschung und Praxis Bd. 29], pp 85-103, Gustav Fischer Verlag, 1987

Fliedner, T.M., W. Nothdurft and K.H. Steinbach (1987)
Blood cell changes after radiation exposure as an indicator for hemopoietic stem cell function.
XIII Annual Meeting of the EBMT, March 1-5, 1987, Interlaken. Bone Marrow Transplantation (in press)

Nothdurft, W., K. Baltschukat and T.M. Fliedner
Untersuchungen über die Kompensationsmechanismen und Regeneration der Hämopoese nach einzeitiger Halbkörperbestrahlung im Tierexperiment an Hunden.
Invited Paper, European Symposium 1987 on Half Body and Total Body Irradiation, 30.9.-3.10.1987, Dresden/DDR (Radiobiol.-Radiother., in press)

2. Short communicatiors, abstracts

Baltschukat, K., W. Ncthdurft and T.M. Fliedner (1987)
Acute and residual haematological effects of partial body irradiation of dogs: I. Irradiation of the lower part of the body with a single myeloablative dose of 11.7 Gy.
Int. J. Radiat. Biol. 51, p. 747 [Abstract]

Baltschukat, K., W. Nothdurft and T.M. Fliedner (1987)
Unilateral exposure of dogs to 300 kV X-rays: hematological effects of an inhomogeneous dose distribution assessed by granulocyte/macrophage progenitor cell (GM-CFC) determinations.
8th International Congress of Radiation Research, 19.-24.7.1987, Edinburgh, Abstracts Part E No. E40-8V, p. 284

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 disintegration. 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:

With respect to the functional characterization of the hemopoietic supportive environment it was of importance to determine the radiosensitivity of the progenitor cells, i.e. CFU-F. Furthermore, the long-term effects of moderate doses of radiation on the bone marrow stroma and on the CFU-F compartment and their possible impact on the hemopoietic tissue were studied in dogs which had been exposed to an inhomogeneous total body irradiation (TBI). Further emphasis was paid to studies directed to the development of long-term canine bone marrow cultures using different approaches.

III. Progress achieved:

.. Methodology

To obtain cell suspensions with appropriate concentrations of CFU-F for the studies of their radiation response bone marrow samples were centrifuged at 400 g for 10 min. The buffy coat cells containing the CFU-F were collected and irradiated with different radiation doses in the range from 0.4 Gy to 10 Gy using 280 kVp X-rays (HVL = 2 mm Cu; dose rate 0.70 Gy/min). Colony scoring was performed after 14 days.

The possible long-term effects of moderate radiation doses of TBI on the stroma and its progenitor cell compartment, CFU-F, on the one hand and the hemopoietic tissue on the other were studied in a group of dogs which had been exposed to TBI with a rather inhomogeneous dose distribution. Bone marrow specimens including pieces obtained from the ribs were used for determinations of the total cell numbers and of the granulocyte/macrophage progenitor cells (GM-CFC) and the CFU-F.

The various approaches to establish long-term canine bone marrow cultures are based on the culture system according to Dexter. First, different bone marrow preparations were used to establish an adherent cell layer in the culture flashs as a hemopoietic supportive environment. Second, different culture conditions especially with respect to the serum components in the medium were tested for their influence on the maintenance of hemopoietic activity.

2. Results

a) Reproducible survival data for canine bone marrow CFU-F were obtained in all of the experiments performed. Obviously survival was related to the radiation dose not in a simple exponential fasnion. An appropriate fit to the empirical data was obtained by means of a multitarget-model $N/No = 1 - (1 - e^{-D/D_o})^n$ though there was a clear indication of a certain slope in the dose range below 1 Gy. The survival curve parameters obtained were $D \sim 2{,}45$ Gy and $n \sim 1{,}3$.

b) Under the inhomogeneous exposure conditions applied quite different radiation doses were obtained in the bone marrow of the ribs on the left side of the body or the right side, i.e. approximately 4 Gy or 1 Gy. The most essential findings obtained 1 year after the exposure were as follows: The number of CFU-F obtained from the bone marrow of a 1 cm piece of a rib showed no differences between the ribs from the left side and the right side. However, the total number of hemopoietic cells was significantly lower in the bone

marrow of the ribs that had received 4 Gy when compared to the contralateral ribs that were irradiated with 1 Gy. A similar difference by a factor of 1.7 could be detected in the GM-CFC numbers.

c) Different bone marrow preparations were tested for their suitability to establish adherent cell layers. The best results with a layer maintenance of more than 10 weeks were obtained when bone marrow was scraped out from the bones and cultured directly without any prior separation, or when buffy coat cells were cultured that were collected from bone marrow cell suspension after centrifugation at 400 g for 10 min. On the other hand, nucleated cell suspensions obtained from bone marrow aspirates by means of sedimentation in the presence of dextran or by density gradient centrifugation (Lymphoprep) produced poor layers if any. The maintenance of hemopoietic activity in the cultures as assessed by the presence of GM-CFC was clearly dependent on the serum constituents of the culture medium and further supplementation. At least GM-CFC could be found up to 8 weeks after preparation of the cultures, when the culture medium was supplemented with dog and horse serum (10% each) and 10 hydrocortisone. No further improvement could be obtained with culture flasks prepared with an extracellular matrix.

3. Discussion

The results obtained from the irradiation experiments on the CFU-F indicate that these progenitor cells are relatively radioresistant when compared to hemopoietic stem cells or progenitor cells. Furthermore, the presence of a shoulder is indicative of some capacity for accumulation of sublethal damage. The findings that in the total body irradiated dogs no differences could be detected between the GFU-F numbers in the ribs after different radiation doses show that the initial cell loss in this compartment must have been compensated in part at least within the 1 years period. On the other hand, there is clearly some late damage present in the hemopoietic activity in the bone marrow that received a radiation dose of 4 Gy. This is obviously not related to the CFU-F. From the results obtained from the long-term bone marrow cultures it becomes evident that in comparison to previous findings a significant improvement could be obtained by supplementation with certain sera. However, within the culture period there is obviously a loss of some cell types that are required for the maintenance of hemopoiesis.

IV. Objectives for the next reporting period:

The further research directed to the functional and radiobiological characterization of the stroma and its progenitor cells will be the following: The response of the CFU-F to irradiation in vitro will be studied in a more detail including the capacity for repair of SLD and PLD. Emphasis will be laid on further improvement of long-term canine bone marrow cultures using specific growth factors. Such cultures will be prepared from the bone marrow of total body and partial body irradiated dogs to discriminate between stromal defects and damage to hemopoietic cells.

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

Calvo, W., T.M. Fliedner, E.W. Herbst, E. Hügl and B. Bödey (1987)
Degeneration and atrophy of the thymus of lethally irradiated dogs, rescued by transfusion of cryopreserved autologous blood leukocytes.
Exp. Hematol. 15, 1171-1178

Calvo, W., J.W. Hopewell, H.S. Reinhold, A.P. van den Berg and T.K. Yeung (1987)
Dose-dependent and time-dependent changes in the choroid plexus of the irradiated rat brain.
Brit. J. Radiol. 60, 1109-1117

Bödey, B., W. Calvo, O. Prümmer, T.M. Fliedner and M. Borysenko (1987)
Development and histogenesis of the thymus in dog. A light and electron microscopical study.
Developmental and Comparative Immunology 11, 227-238

Fliedner, T.M. and K.H. Steinbach
Repopulating potential of hematopoietic precursor cells.
Blood Cells Symposium, 26.-28.10.1987, Reisensburg (Blood Cells, in press)

Kreja, L., K. Baltschukat and W. Nothdurft
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. (in press)

Ludwig, R., W. Calvo, B. Kober and W.E. Brandeis (1987)
Effects of local irradiation and i.v. methotrexate on brain morphology in rabbits: early changes.
J. Cancer Res. Clin. Oncol. 113, 235-240

Michel, C., W. Calvo, O. Prümmer and T.M. Fliedner (1987)
Histochemical studies on the pancreas of dogs rescue by fetal liver cell transplantation after lethal total body X-irradiation.
Cellular and Molecular Biology 33, 91-100

Nothdurft, W., K. Baltschukat and T.M. Fliedner
Hematological effects in dogs after sequential irradiation of the upper and lower part of the body with single myeloablative doses.
Radiother. Oncol. (submitted 1987)

Prümmer, O., W. Calvo and T.M. Fliedner (1987)
Variation of treatment conditions alters the outcome of fetal liver transplantation.
Thymus 10, 19-31

2. Short communications, abstracts

Fliedner, T.M. (1987)
Experimentelle Grundlagen der Stammzelltransplantation.
93. Tagung der Deutschen Gesellschaft für Innere Medizin, 2.6. - 30.4.1987, Wiesbaden

Kreja, L., W. Nothdurft and K. Baltschukat (1987)
Long-term canine bone marrow culture.
Meeting of the European Stem Cell Club, April 29-30, 1987, Institute Pasteur, Paris, Book of Abstracts, Abstract No. 33

Nothdurft, W., K. Baltschukat and T.M. Fliedner (1987)
Acute and residual haematological effects of partial body irradiation of dogs: II. irradiation of the upper and lower part of the body with single myeloablative doses separated by an interval of 56 days.
Int. J. Radiat. Biol. 51, 252 [Abstract].

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, Prof. Dr. W. Nothdurft, Dr. L. Kreja, Dr. K.H. Steinbach, A. Ingendaay

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:

As reported last year there are difficulties in collaboration with the Argonne Laboratory due to shortage in marpower. A planed visit to Argonne could not be realized.

The studies with mice using methylnitrosourea and radiation have been successfully finished, the final report is given in a booklet and a final paper. Long-term studies using benzene as chemical leukemogen have been begun.

III. Progress achieved:

The co-leukemogenic effect of low doses of radiation (total body irradiation) with small doses of the chemical leukemogen methylnitrosourea has been clearly documented in T cell leukemogenesis. In protocols with radiation followed by methylnitrosourea, both as single doses, a critical interval was found, at which a high sensitivity for induction of malignancy exists. This was interpreted as relative enrichment of target cells. It was, however, not possible to characterize this target cell futher, too little is known about the bone marrow to thymus interaction and cell flow.

The studies with benzene as inhaltation leukemogen could be started after the establishment of an inhalation chamber for gaseous chemicals with all the necessary control elements. In a first series of experiments three doses of benzene were given for periods of 8 and 16 weeks, respectively. A detailed analysis of the hemopoietic stem cell system is not the objective of this report, but the long-term effects with respect to induction of malignancies. A protocol was found with about 30% of mainly hemo-lymphopoietic tumors up to one year after the end of the exposure period.

IV. Objectives for the next reporting period:

The long-term studies with benzene will be further persued and emphasis will be given to the induction of malignancies and aplasias after combination with radiation. This will include molecular aspects of leukemogenesis and cellular aspects of the microenvironment using the CFU-F technique.

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

The studies are performed in close collaboration with Dr. E.P. Cronkite/Brookhaven, who was as a visiting professor in our laboratory. There is also exchange within the European Late Effects Project Group. During 1988 further contacts will be established, e.g. to the groups at Research Triangle Park in North Carolina.

VI. Publications:

H.J. Seidel: Die Entwicklung von T-Zell-Leukämien. Untersuchungen an der Maus nach einmaliger Gabe von Methylritrosoharnstoff unter Mitarbeit von Ludwika Kreja. Thieme copythek, Stuttgart, 1986

H.J. Seidel: Effects of radiation and other influences on chemical lymphomagenesis. Int. J. Radiat. Biol. 51, 1041-1048, 1987

H.J. Seidel, D. Zinser, M. Pforr, G. Beyvers: Benzene inhalation and hemopoietic stem cells in mice. Exp. Hematol. 15, 518, 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: B16-C-062-UK

Paterson Laboratories
Christie Hospital
and Holt Radium Institute
Wilmslow Road
GB - Manchester M20 9BX

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, R. Gallini, G. Molineux, J. Bierkens, C. Tejero, M.C.Baird, B.I.Lord

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 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 cell hierarchy in the mouse, dog, and human, using various growth factors.
(b) To assess residual haemopoietic injury in the mouse after fractionated gamma-irradiation, down to 0.1 Gy per fraction.
(c) To study the cycling of haemopoietic precursor cells and its regulation in mouse bone marrow after single-dose or repeated irradiation.

III. Progress achieved:

(a) Cultures of γ-irradiated murine bone-marrow cells were grown using different colony-stimulating factors (CSF) to analyse in detail the radiosensitivity of the colony-forming cells (CFC) in the haemopoietic hierarchy. These factors included purified,native or recombinant Interleukin-3 (IL-3), Granulocyte-Macrophage (GM)-CSF, Granulocyte (G)-CSF, Macrophage (M)-CSF and IL-1) (a gift from Professor T M Dexter). IL-1 lacks intrinsic colony stimulating activity itself, but potentiates CSF responses of normally non-responsive immature haemopoietic cells. These CSF's were either used alone or in various combinations to detect the radiosensitivity of different cell populations responsive to these CSF-s.

With unfractionated bone-marrow the Do values ranged from 1.3 Gy (GM-CSF) to 1.0 Gy (M-CSF), all with n = 1. However, when IL-3 was used the Do was 1.2 Gy and the survival curve had a shoulder (n = 1.9). A combination of IL-3 with IL-1 resulted in a Do of the 1.2 Gy with n = 1, hence the IL-1 may somehow reduce the repair capacity of the CFC responsive to IL-3. When the cells were co-treated with M-CSF and IL-1 the Do was again 1.2 Gy with n = 1, indicating that the population stimulated may be the same population recruited by IL-3 with IL-1. The combination of IL-3 with M-CSF and with IL-1 produced a biphasic survival curve with a Do of 1.7 Gy for the resistant population (60%), suggesting that these CSF-s may have stimulated a sub-population of resistant progenitors.These data indicate that the use of different CSF-s can influence the shape of the survival curve for GM-CFC and that the progenitor cells become increasingly more radiosensitive as they progress through development.

Also, we have been investigating the radiosensitivity of human and canine CFC. For human CFC the CSF's used were 5637 conditioned medium (CM), rIL-3, rGM, and rG. For the dog, phytohaem-aglutinin (PHA)-CM, irradiated dog serum, and all the human CSF's are being used as sources of CSF. Using 5637-CM (GM-CSF, G-CSF, and IL-1) we have obtained a Do of 1.3 Gy, n = 1, for human CFC, and for canine CFC Do = 0.5 Gy, n = 1, using PHA-CM.

(b) Groups of mice were given 15 fractions (5 per week) whole-body gamma-irradiation (0.064 Gy per minute) using equal doses per fraction, and the dose per fraction ranged from 0.7 down to 0.1 Gy per fraction. Other groups received one course of 15 fractions, followed after a recovery interval of 3 weeks, by a second course of 15 fractions.

At day 3 after the last of 15 fractions, there was a dose-dependent reduction in the number of CFU-S per femur, characterised by a Do value of about 3 Gy. There was marked recovery by 3 weeks to about 50% of the age-matched controls, with little dose-dependence being demonstrated. There was little further recovery, and by 9-12 months numbers of CFU-S were still subnormal (60-80%) even after the smallest total doses used of 1.5 Gy.

GM-CFC were less affected than CFU-S at day 3 after the last dose. Recovery improved to 6 months and then later declined, and by 12 months the levels of CFU-S and GM-CFC were similar. Values of CFU-S per colony were reduced at 3 days and at 3 weeks after the last dose.Later there was recovery but by 6-12 months after the highest total dose of 10.5 Gy the values were about half normal.

CFU-F were less affected than the CFU-S and GM-CFC at day 3 after the last dose, and there was good recovery in the longterm after the lower doses. The data for CFU-S/ossicle were more scattered, but overall they showed a similar pattern to that seen for CFU-F.

In the longterm the levels of CFU-S after the higher accumulated doses were slightly lower suggesting greater injury by higher accumulated doses. Stem-cell quality, assessed by CFU-S per colony, showed a consistent reduction after the highest accumulated dose of 21 Gy delivered in 30 fractions.

(c) The rate of cycling of stem cells (CFU-S) and granulocyte-macrophage colony-forming cells (GM-CFC) was measured in the femoral marrow of mice at various times after whole-body irradiation with 1.5-10 Gy or 4 doses of 4.5 Gy (each separated by 3 weeks.)

A dose-dependent increase in the cycling rate of both CFU-S and GM-CFC was observed which was similar at all times of assay between 3 weeks and 36 months.

This invariance with time was in contrast to in controls, where for GM-CFC the cycling rate declined unexpectedly with increasing age of the mice. This latter observation is of importance in the use of cycle-specific cytotoxic drugs where less effect on this important cell population may be found in older animals, and possibly in man.

The cycling of CFU-S and GM-CFC was also increased at 6-10 months after 4 doses of 4.5 Gy, and the femoral content of these precursor cells was reduced more than after single doses. In addition, when assessing separately the central core of marrow and the peripheral cylinder inside the bone shaft, it was found that the concentration of CFU-S was similar in both regions. This is in contrast with the higher concentration found in marginal than in axial marrow in controls. Assessment of the level and production of a stimulator of CFU-S cycling showed that the level was elevated in both axial and marginal marrow fractions, but the rate of production of stimulator was much reduced.

IV. Objectives for the next reporting period:

(a) to complete the comparison of the radiosensitivity of precursor cells in the haemopoietic lineages in the mouse, dog, and human, using different 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.

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

VI. Publications:

Molineux, G., Testa, N.G., Hendry, J.H., Schofield, R. (1987) The radiation sensitivity of the haemopoietic microenvironment: effect of dose-rate on ectopic ossicle formation. Radiotherapy and Oncology, 10: 157-161.

Wang, S.B., Hendry, J.H., Testa, N.G. (1987) Sensitivity and recovery of stromal progenitor cells (CFU-F) in mouse bone marrow given γ-irradiation at 0.65 Gy per day. Biomedicine and Pharmacotherapie, 41: 48-50.

Gallini, R., Hendry, J.H., Molineux, G., Testa, N.G. (1987) The effect of low dose-rate on longterm recovery of haemopoietic and stromal progenitor cells in γ-irradiated mouse bone marrow. Radiation Research (in press).

Gallini, R., Hendry, J.H., Molineux, G., Testa, N.G. (1987) Residual haemopoietic injury in the mouse after fractionated gamma-irradiation, down to 0.1 Gy per fraction. Radiotherapy and Oncology (submitted).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no.: BIG-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
Churchill Hospital Research Inst.
University of Oxford
Headington
GB - Oxford OX3 7LJ

Telephone number: 0865-64841

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

I. Objectives of the project:

The pathogenesis of early and late radiation damage to the skin will be studied in the pig. The results obtained from experiments in this species are more likely to provide data which may be more readily extrapolated to man.

II. Objectives for the reporting period:

Late radiation damage to the skin, as characterised by dermal atrophy,

Late radiation damage to the skin, as characterised by dermal atrophy, may have important implications for radiological protection. Serial histological studies have been carried out over the time course of the development of radiation-induced atrophy of pig skin. In the present report the details of the changes in the numbers of fibroblast nuclei are given. These are compared with gross estimates of the degree of dermal atrophy, as obtained from measurement of the linear dimensions of the irradiated area and with measurements of dermal thickness 'in vivo' using ultrasound echo location. These findings are compared with earlier estimates of changes in dermal blood flow.

III. Progress achieved:

The time-related changes in the density of fibroblast nuclei were studied after irradiation of the skin with a single dose of 15.4Gy of x-rays. Counts of nuclei were made in the reticular dermis of histological sections obtained from samples of skin taken when animals were killed at 6 - 104 weeks after irradiation. Counts of fibroblast nuclei were also made from areas of unirradiated skin from the same animals.

In unirradiated pig skin there was only a small decline in the mean number of fibroblast nuclei, per unit area, with increasing age (Fig. 1). This would suggest a rise in the total number of fibroblasts with age, the thickness of the dermis increased from 1.3mm at 13 weeks, to 1.8mm at 26 weeks, and to 3.6mm in mature animals, aged ≥65 weeks. Growth was attributed to an increase in the diameter of the collagen bundles. Following irradiation with a single dose of 15.4Gy there was no significant change in the fibroblast density for the first 12 - 14 weeks. This suggests that this radiation dose has no effect on the increase in total fibroblast number associated with growth over this period. After 14 weeks the fibroblast density declined, reaching plateau value of ~60% of that of unirradiated skin between 26 and 40 weeks after irradiation. Ultrasound measurements of dermal thickness have shown a similar pattern of changes in dermal thickness.

At intervals of 26, 40, 52 and 104 weeks after irradiation the dose-related changes in the number of fibroblast nuclei in the reticular were studied. All data points could be fitted by the same dose-effect curve. This curve had an initial shoulder region after which the fall in the density of fibroblast nuclei was linearly related to the dose (Fig. 2).

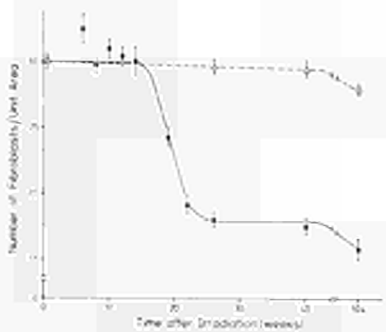

Fig. 1: Time-related changes in the density of fibroblast nuclei (±SE) after a single dose of 15.3Gy of (●) x-rays (△ - unirradiated skin)

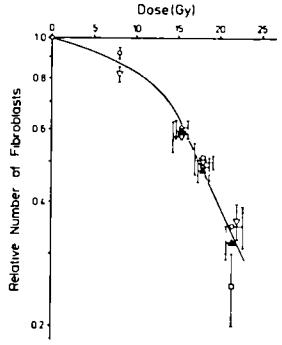

Fig. 2: Dose-related change in the relative number of fibroblast nuclei (±SE) at 26 (○), 40 (▲), 52 (□) and 104 weeks (▽) after irradiation

These changes in the number of fibroblast nuclei are not seen as a primary effect of radiation, since it would appear to follow a sequence of change in the dermal vasculature. These vascular changes culminate in a dose-related reduction in blood flow at 12 weeks after irradiation (Moustafa & Hopewell, Brit. J. Radiol. 52, 138-144, 1979). This dose-related reduction in dermal blood flow at 12 weeks would appear to be similar to the decline in fibroblast nuclei at 26 weeks (Fig. 3). Two other parameters, the reduction in linear field dimensions after 6 - 12 months and dermal thinning measured at 52 weeks would appear to show a less severe degree of damage. However, when the percentage reductions in these linear dimensions are corrected to represent the reduction in tissue area or volume, they are more comparable with the fibroblast changes. Thus the initial reduction in dermal blood flow at 12 weeks is a useful predictor of the subsequent severity of dermal injury.

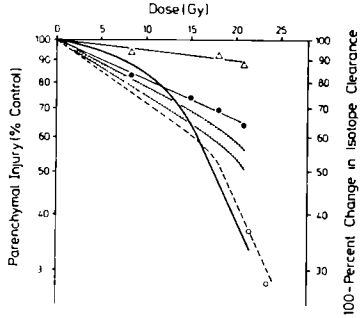

Fig. 3: Comparison of dose-related changes in fibroblast density (-) reduction in blood flow at 12 weeks (○), linear field contraction after 26 - 52 weeks (△) and dermal thickness at 52 weeks (●). The reduction in the linear parameters have been corrected with respect to tissue area and volume (hatched area)

IV. Objectives for the next reporting period:

Preliminary observation would suggest that lymphatic function is impaired from as early as six weeks after irradiation with a single dose of 18Gy of x-rays. This would appear to be the earliest detectable change in the dermis, preceding the reduction in blood flow by several weeks. This relationship between impaired lymph flow, the development of dermal oedema and a reduction in dermal blood flow requires further investigation.

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

VI. Publications:

Morris, G.M. and Hopewell, J.W. (1987): Cell population kinetics in pig epidermis : further studies. Cell Tissue Kinet. 20, 161-169

Hopewell, J.W. (1987): The role of the vasculature in normal tissue responses. Radiation Research Proc. 8th I.C.R.R. (Edinburgh) Eds. E.M. Fieldon, J.F. Fowler, J.H. Hendry and D. Scott (Taylor Francis, London) vol. 2, pp779-794

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

I. Objectives of the project:

Concern has been expressed as to the adequacy of existing radiological protection guidelines for the skin. The studies will provide information on the early and late effects of irradiation at different dose-rates from sources of varying size and energy. It is hoped that this data will provide an adequate basis for an improvement to the present radiological protection guidelines.

II. Objectives for the reporting period:

In last year's report information was provided on the late atrophic changes induced in pig skin by irradiation with thulium-170 source of 2 - 19mm diameter. Comparable data are now available for smaller individual, 0.1 - 1.0mm, diameter sources and for 2mm sources arranged in the form of a multi-source assay.

III. Progress achieved.

In a previous report results were presented for the dose-related changes in dermal atrophy in pig skin assessed two years after irradiation from thulium-170 sources of 2 - 19mm diameter. Measurements were made from 5μm thick histological sections and the degree of dermal atrophy was expressed as a ratio of the irradiated to the unirradiated dermal thickness. These results showed that after a threshold skin surface dose of ~20Gy the average severity of atrophy increased with dose, reaching a maximum 35% reduction in dermal thickness after doses ⩾60Gy. No differences in response were seen between 2mm and 19mm diameter sources.

Comparable measurements have now been made after the irradiation of skin sites with very small sources of 0.1, 0.5 and 1mm diameter. The studies with a 2mm source were repeated. A reduction in the relative thickness of the dermis was seen after a threshold dose of ~40Gy. The maximum reduction in the thickness of the dermis of 15% being seen after doses >120Gy. The results obtained after irradiation with 2mm diameter sources were comparable with those reported previously.

In an alternative analysis, these results were converted into quantal data by assessing the proportion of skin sites irradiated, at each dose level, that exceeded a given severity of reaction. A ⩾20% reduction in dermal thickness was selected as this might reasonably be termed 'detrimental' in terms of the present radiological protection guidelines. The resulting dose-effect curves were fitted by probit analysis and from these curves the ED_{50} (±SE) values were obtained (Fig. 1). For 2, 9 and

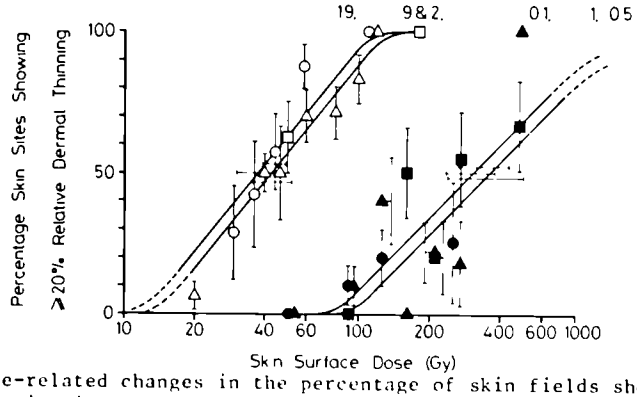

Fig. 1: Dose-related changes in the percentage of skin fields showing a >20% reduction in dermal thickness at 104 weeks after irradiation with thulium-170 sources of 19, 9, 2, 1, 0.5 and 0.1mm diameter. Error bars ±SE

19mm diameter sources an ED_{50} of ~40Gy was obtained, significantly lower than that for the three smaller sources (~350Gy). This separation of the dose-effect curves with respect to very small and larger sources was also characteristic of the acute responses of the skin to radiation; however, in this instance the areas irradiated from the 2mm source were more comparable with the 0.1 - 1.0mm diameter source and not those of larger diameter.

The late response to a single 2mm diameter source has also been compared with that from 2mm sources arranged in the form of an array in a 20mm x 40mm field: 8 sources (in 2 rows of 4) separated by 8mm or 32 sources (in 4 rows of 8) separated by 3mm, were used. Measurements of dermal thickness were made at the central axis of each individual source and also at points midway between two sources. The dose-related changes in relative dermal thickness for a single 2mm diameter source and the arrays of 2mm sources are shown in Fig. 2. For the array of 8 sources the maximum reduction in dermal thickness was comparable with that seen after irradiation with a single 2mm diameter source. However, for the array of 32 sources the maximum severity of dermal thinning was surprisingly reduced.

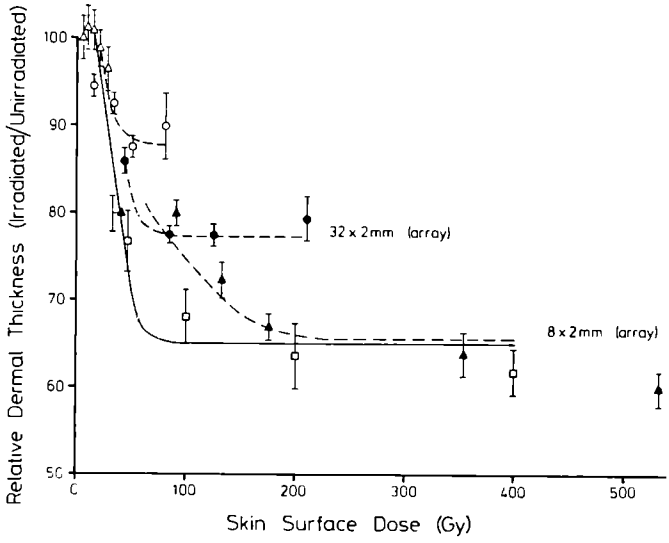

Fig. 2: Dose-related changes in the relative thickness of the dermis 104 weeks after irradiation with a single 2mm source (□) and with arrays of 8 (△ ▲) and 32 (○ ●) 2mm sources of thulium-170 (▲ ● skin surface doses under the sources; △ ○ dose between the sources; Error bars ±SE)

IV. Objectives for the next reporting period:

The studies of the acute and late response of the skin following irradiation from strontium-90 source of differing dose-rate, that were initiated in the present reporting period, will be continued.

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

Dr. J. Wells and Dr. M.W. Charles
Radiobiology Laboratory
Health Physics Research
CEGB Berkeley Nuclear Laboratories
Berkeley
Gloucestershire GL13 9PB

Dr. J.E. Coggle
Department of Radiobiology
Medical College of St. Bartholomew's Hospital
Charterhouse Square
London EC1M 6BQ

VI. Publications:

Wells, J., Hopewell, J.W., Charles, M.W. and Coggle, J.E. (1987): Accidental exposure of skin to beta emitters : dose distribution and biological effects (abstract). Proc. 8th Int. Cong. Radiat. Res. Edinburgh. Eds. E.M. Fielden, J.F. Fowler, J.H. Hendry and D. Scott. Taylor and Francis (London) vol. 1, p258

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-C-065-F

Centre International
de Radiopathologie
B.P. n° 34
F - 92260 Fontenay-aux-Roses

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: 1-654.49.29

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.

CONTRAT DE RECHERCHE
N° B16-065-F

RAPPORT INTERMEDIAIRE DECEMBRE 1987

TITRE DU CONTRAT DE RECHERCHE :

- Effets non stochastiques de l'irradiation de l'homme : diagnostic, pronostic et traitement des irradiations aigües accidentelles

- Non stochastic effects of irradiation in man : diagnosis, prognosis and treatment of acute radiation injury.

LISTE DES PROJETS :

1 - DIAGNOSTIC ET TRAITEMENT DES RADIOLESIONS CUTANEES :

- Evaluation biophysique, biochimique et biologique des dommages à la peau et aux tissus sous jacents après irradiation accidentelle. Amélioration des protocoles thérapeutiques.

- Biophysical, biochemical and cytological diagnosis of damage to skin and underlying tissues after accidental exposure. Improvement of therapeutic protocols.

2 - DIAGNOSTIC ET TRAITEMENT DE L'IRRADIATION GLOBALE.

- Biological indicators of global irradiation

1 - DIAGNOSTIC ET TRAITEMENTS DES RADIOLESIONS CUTANEES

1-1 : EXAMENS PARACLINIQUES

- L'imagerie par résonnance nucléaire (IRM) a été appliquée à 1 sujet irradié sur la face dorsale des doigts de la main, par des photons γ (^{192}Ir).
Les images ont été obtenues à Orsay (France) dans le Service des Drs Lhoste et Cauzade à deux niveaux de fréquence et d'intensité de champs :

a - Image à très haute fréquence, permettant de séparer les raies de résonnance des protons de l'eau de celles des protons des tissus adipeux (CH2)

b - Image à fréquence moyenne
Les images obtenues n'ont pas apporté d'information nouvelle par rapport à la clinique sur l'extension de la lésion, en raison du niveau modéré d'irradiation et de sa localisation sur la face dorsale peu épaisse des doigts de la main.
Cette observation montre certaines limitations à la valeur diagnostique de l'IRM.

- L'observation microscopique des capillaires du lit inguéal (vidéocapillaroscopie) que nous pratiquons depuis 1983 a été grandement améliorée en 1987 par l'adjonction d'un système vidéo SONY . Le stockage sur magnétoscope au standard professionnel U.matic d'un nombre élevé d'images à partir d'une mini caméra haute sensibilité SONY permet à posteriori de sélectionner techniquement les plus significatives pour l'exploration et permet en outre d'envisager des mesures de perfusion ainsi que le développement d'épreuves physiologiques.

- Les premiers documents encourageants obtenus à l'aide de la caméra microondes prototype de seconde génération ont conduit les promoteurs à poursuivre leurs efforts sur l'amélioration des transducteurs et la simplification du principe de la mesure tant pour diminuer le temps de réponse et l'influence des champs parasites crées par la matrice des transducteurs elle-même que pour affiner la résolution de l'image finale. Une caméra de troisième génération actuellement à l'essai parait améliorer sensiblement la résolution géométrique et temporelle des élements constitutifs de l'image.

1-2 : ETUDE DE LA CINETIQUE CELLULAIRE DES TISSUS IRRADIES

Des marqueurs de la prolifération cellulaire ont été introduits :
a) analyse du cycle par cytométrie en flux (CMF)
b) anticorps Ki67, contre un marqueur des cellules en cycle
c) marquage au BudR de la phase S
La comparaison de ces différents marqueurs de prolifération est en cours pour en déterminer l'intérêt pratique en cas d'irradiation accidentelle.
Seule la troisième technique donne un résultat fonctionnel et peut se pratiquer sur tissu vivant. Elle a été mise au point sur des lignées cellulaires en culture et a été utilisée également sur des cultures de lymphocytes stimulés par la PHA. L' incorporation de BudR est visualisée par immunomarquage révélé à la péroxydase. Les lames sont lues de manière automatique avec un analyseur d'image (SAMBA).

L' analyse du contenu en ADN des cellules est effectué par cytométrie en flux. Ainsi il est possible de déterminer la distribution dans les différentes phases du cycle. La technique est applicable à des échantillons frais, congelés voire même fixés. L' immunomarquage avec l' anticorps Ki-67 est complémentaire par rapport aux deux techniques décrites précédemment. Son utilité repose essentiellement sur la possibilité d' utilisation sur échantillons congelés, ainsi que pour l'estimation de la proportion de cellules quiescentes par rapport à la CMF.

1-3 : ETUDE DU RÔLE DES FACTEURS DE CROISSANCE SUR LA FIBROSE RADIOINDUITE.

En collaboration avec le Pr D. Barritault (Université de Créteil) un dosage des récepteurs de FGF (fibroblast growth factor) utilisant du ^{125}I-FGF a été mis au point et testé, en collaboration avec le Laboratoire de Radiologie Animale de Jouy en Josas (Dr Daburon) sur des cultures de fibroblastes de peau témoin ou irradiée. Les résultats ne sont pas encore complets.
Dans le même but d'évaluer le rôle des facteurs de croissance et de leur récepteur sur la cinétique des tissus irradiés, un dosage du récepteur d'EGF a été mis au point et utilisé sur des cultures de cellules.
Pour évaluer le rôle des agents inducteurs de fibrose comme le TGF ß (transforming growth factor ß) des sondes ADN et ARN ont été produites à partir d'une partie clonée du gène du TGF ß (Dr DERYNCK, GENENTECH) pour la détection de la surexpression éventuelle de ce gène après irradiation. Des essais d'hybridation in situ sont en cours.
Parallèlement, on a entrepris la préparation d'anticorps, d'abord polyclonaux, contre une partie C-terminale peptidique du TGF-ß obtenue par synthèse peptidique. Les antiserums obtenus sont en cours de titrage.

PERSPECTIVES
Etude du rôle des facteurs de croissance dans la régénération tissulaire après irradiation.

2 - IRRADIATION GLOBALE

2-1 : NATURE ET REPARATION DES DOMMAGES GENOTOXIQUES RADIOINDUITS.

OBJECTIF
Le but de ce travail est de savoir si l'exposition préalable à une irradiation globale (^{60}Co) modifie la radiosensibilité des lymphocytes des sujets irradiés. Les aberrations chromosomiques et la capacité de réparation de dommages induits par une irradiation ultérieure "in vitro" sont les deux paramètres de radiosensibilité testés. La partie de l'étude concernant l'évaluation des aberrations chromosomiques est faite dans le laboratoire de Physiologie expérimentale (M.T. DOLOY, SPE - IPSN - CEA FAR).

MATERIEL BIOLOGIQUE
Dans le cadre de cette étude, nous avons testé les prélèvements de sang issus de 12 singes qui ont été exposés à une irradiation globale (^{60}Co). Six singes ont reçu une dose de 8 ou 10 Gy d'irradiation fractionnée (groupe I) 3 singes ont reçu 2 Gy d'irradiation fractionnée (groupe II) et 3 autres ont été exposés à 2 Gy d'irradiation aigue (groupe III).

Actuellement, une étude en cours inclut le test de prélèvements de sang issus de patients cancéreux avant et après traitement séquentiel de radiothérapie locale et chimiothérapie.

METHODE

Les lymphocytes isolés sont irradiés "in vitro" (6 Gy ^{137}Cs). Le taux des dommages initiaux radioinduits et leur réparation sont mesurés par la méthode spectrofluorimétrique F.A.D.U. (Fluorimetric Analysis of DNA Undwinding).

RESULTATS

a - singes irradiés.

Les réponses des singes irradiés globalement sont comparées à celles de singes témoins testés simultanément à cause de la variabilité individuelle observée. Une analyse statistique des résultats (test de Wilcoxon pour séries appariées) a été conduite pour chacun des 3 groupes de singes, la répartition dans ces groupes étant basée sur la dose totale reçue, et le mode d'irradiation "in vivo". En résumé, cette analyse a permis de constater : (1) aucune différence significative des taux de dommages induits par l'irradiation "in vitro", (2) une capacité de réparation diminuée chez les singes qui ont reçu une dose d'irradiation fractionnée de 8 ou 10 Gy (Groupe I), (3) une capacité de réparation similaire à celle des singes témoins dans le cas des singes qui ont été exposés à 2 Gy, la cinétique de réparation semblant être plus lente dans le cas d'une irradiation aigue de 2 Gy (Groupe III).

Nous n'avons pu mettre en évidence de corrélation entre la modification de la radiosensibilité chromosomique et celle de la capacité de réparation chez le singe irradié.

b - patients cancéreux.

L'étude est en cours. Les premiers résultats semblent indiquer que la réparation de dommages de l'ADN des lymphocytes de ces patients prélevés avant tout traitement est plus faible que celle de sujets normaux. Cette différence entre les réponses des lymphocytes de 25 patients et de ceux de sujets normaux testés simultanément, est statistiquement significative (test de Student pour séries appariées $p<0.05$). Le test des lymphocytes de patients traités en radiothérapie ou chimiothérapie est en cours. L'évaluation des aberrations chromosomiques induites par une irradiation ultérieure "in vitro" sur les mêmes prélèvements est en cours en collaboration avec le laboratoire de Mme DOLOY.

2-2 : ETUDE DE L'IRRADIATION SUR LE TISSU HEMATOPOÏETIQUE : FACTEURS DE LA FIBROSE MYELOÏDE.

Des sondes et des anticorps permettant de mesurer l'expression du TGF ß ont été préparés pour l'étude de la fonction du mégakaryocyte et des plaquettes dans l'induction de la fibrose des tissus hématopoïétiques. Ces réactifs seront utilisés soit directement sur des moelles fibrotiques soit sur les colonies de mégakaryocytes développées par le Dr Martyré à l'Institut Curie.

2-3 : ETUDE DE LA RESTAURATION DE LA FONCTION MEDULLAIRE APRES IRRADIATION.

Les problèmes rencontrés lors des greffes de moelle osseuse suite aux irradiations reçues pendant l'accident de la centrale nucléaire de Tchernobyl impliquent que des études complémentaires soient menées pour la mise au point de nouvelles techniques de greffes adaptées aux conditions particulières rencontrées lors des accidents (irradiations larges mais souvent hétérogènes, dosimétrie approximative, compatibilité avec le donneur difficile à établir).

Depuis quelques années se sont développées des techniques de culture de moelle osseuse à long terme présentant un grand intérêt dans le domaine de la radiopathologie.
Ces cultures ont permis de mettre en évidence l'importance du stroma pour la reprise de l'hématopoïèse à la suite d'un traitement et ont probablement, de ce fait, une grande valeur pronostique. Elles ont également été utilisées en thérapeutique pour réaliser des greffes après culture. Chez la souris, les cultures de moelle de ce type peuvent se maintenir et s'amplifier sur plusieurs mois . Ceci n'est pour l'instant pas le cas dans l'espèce humaine où les cultures périclitent après 10 semaines.

Une meilleure connaissance de l'interaction entre stroma médullaire et cellules souches hématopoïétiques et l'utilisation de facteurs de croissance spécifiques devraient autoriser une amplification des cultures qui est nécessaire à l'application thérapeutique de la technique à la radiopathologie clinique, dans la mesure où dans les irradiations non homogènes étendues, elle devrait permettre de reconstituer la moelle grâce à la réinjection des cellules cultivées et amplifiées in vitro à partir d'un petit prélèvement d'un territoire sain de moelle.

Dans cette optique, une étude corrélant l'effet des traitements à la qualité et la quantité des cellules stromales aptes à créer des foyers lors des cultures, mais aussi à l'hématopoïèse in vitro est menée dans le service. Des prélèvements de moelle pour mise en culture sont récoltés de façon séquentielle chez des malades au cours des traitements (irradiation et/ou greffe). La partie quantitative, c'est à dire l'étude de prolifération cellulaire , point fondamental de ce travail, se fait par des techniques de clonogénicité cellulaire longues et lourdes et nous tentons de mettre au point des techniques plus légères et plus rapides basées sur la modulation de l'oncogène c-myc (marqueur de la prolifération) au cours de la multiplication cellulaire. La partie qualitative de ce travail passe par la reconnaissance, au moyen d'anticorps, des marqueurs de l'origine cellulaire et du niveau de différenciation des cellules stromales et des précurseurs hématopoïétiques.

PROTOCOLES
a - Autogreffe.
Une étude est en cours sur des patients porteurs de neuroblastomes dont la moelle est étudiée avant traitement, en cours et après irradiation corps entier, avant et après autogreffe de moelle, pour essayer de définir l'intérêt thérapeutique diagnostique et pronostique des cultures de moelles à long terme (34 patients + 6 contrôles).

b - Allogreffe
- Une étude est en cours sur des patients atteints d'aplasie médullaire dont la moelle est étudiée avant et après allogreffe (21 patients + 21 contrôles)

c - Radiopathologie appliquée
Des cultures à long terme de moelle et des études de clonogénicités des cellules souches hématopoïétiques circulantes ont été effectuées sur deux patients de radiopathologie (1 irradiation globale protractée estimée à 2 Gy datant de 7 mois, 1 irradiation globale protractée estimée à 14 Gy datant de 9 ans).

RESULTATS
Le protocole (a) a souligné l'impossibilité dans l'état actuel des recherches d'utiliser les cultures de moelle à long terme à des fins thérapeutiques directes mais a mis en évidence l'intérêt diagnostique et pronostique de ces expériences . Le protocole (b) a montré que dans les aplasies étudiées il n'y a pas atteinte du stroma mais que seule l'hématopoïèse est touchée.
L'étude des patients (c) irradiés accidentellement n'a pas permis de souligner une différence nette sur les cultures établies par rapport à nos controles. l'expérience n'est toutefois pas achevée.

PERSPECTIVES
Les études (a) et (b) vont se poursuivre en étudiant l'action du facteur de croissance des granulocytes-macrophages administré en cours de greffe (essai de phase II), traitement d'un grand intérêt en Radiopathologie. D'autre part on essayera d'amplifier les cultures de moelles osseuses par de nouvelles techniques (facteurs de croissance, cultures en trois dimensions, milieu défini). Une étude sur modèle animal est également prévue.

PUBLICATIONS

- Intérêt des cultures de moelles osseuses à long terme en culture humaine. D. THIERRY, Séminaire Mars 1987. Hôpital St Louis.

- Long term bone marrow culture in metastatic neuroblastoma. D.THIERRY, P. VALIDIRE, M. HARDY, H. MAGDELENAT, J. M. ZUCKER. Soumis pour publication à Lancet.

- Long term bone marrow cultures in the treatment of aplastic anemia. F. VARRIN, D. THIERRY, M. BENBUMAN, A. DEVERGIE, H. MAGDELENAT, E. GLUCKMAN, accepté pour publication et Séminaire European Bone Marrow Transplantation Congress 88 .

- A protein of halobacterium halobium immunologically related to v-myc gene product. K. BEN-MAHREZ, B. PERBAL, C. KRICEVE-MARTINERIE, D. THIERRY, and M. KOHIYAMA, accepté pour publication à FEBS letters.

- Detection of circulating antibodies against c-myc protein in cancer patients serum. K. BEN-MAHREZ, D. THIERRY, I. SOROKINE, A. DANNA-MULLER, and M. KOHIYAMA, soumis à B.J. of Cancer.

- Fluorimetric analysis of DNA strand breakage and repair kinetics. Application to radiotoxicology. O. RIGAUD, H. MAGDLENENAT. Accepté mai 87 - à paraître dans : New Trends in genetic Risk Assessment - Nice march 9-11, 1987 (Rhone-Poulenc Santé Eds)

- Chromosomal aberrations and DNA repair ability of in vitro γ irradiated white blood cells of monkeys : effect of a previous total body irradiation. G. GUEDENEY, O. RIGAUD, I. DURANTON, J.L. MALARBET, M.T. DOLOY, and H. MAGDELENAT. (Soumis pour publication)

CONGRES

- Modification of "in vitro" radiation response after total body irradiation in monkeys II DNA damage and repair. O. RIGAUD, G. GUEDENEY, I. DURANTON, M.T. DOLOY and H. MAGDELENAT. Eight International Congress of Radiation Research 19-24 July 1987.

Title of the project no.: B 160065 F

Modifications cellulaires et biochimiques du sang après irradiation corporelle totale.

Head(s) of project:

Pr J. DUTREIX
Pr J.M. GOSSET

Scientific staff: Laboratoire de radiobiologie cellulaire.
(Drs MALAISE - GUICHARD - GIRINSKI)

I. Objectives of the project:

- Etude des modifications des deux types de cellules, granulocytes et lymphocytes pour lesquelles les variations sanguines sont observées pour préciser la cinétique et la relation avec la dose après irradiation à but thérapeutique.
- Etude des modifications biochimiques dans le sang afin de sélectionnner les dosages significatifs, d'en préciser la cinétique de variation et la relation avec la dose.
- Etude de la relation des effets observés avec le passé hématologique des malades
- Etude plus approfondie de certains mécanismes, en particulier afin d'expliquer le pic d'hyperleucocytose.

II. Objectives for the reporting period:

- Etude préliminaire de certains enzymes et hormones.
- Sélection des patients retenus en fonction des modalités d'irradiation.
- Mise en place des protocoles.

III. Progress achieved :

- Les irradiations totales et subtotales retenues pour l'étude sont :
  - (STBI) : Irradiation corporelle totale en 1 séance à faible débit de dose 10 Gy/4 h (débit de dose moyen = 4,2 cGy/min.)
  - (HTBI) : Irradiation corporelle totale hyperfractionnée : 11 x 1,35 Gy en 4 jours (3 séances par jour).
  - (FTBI) : Irradiation corporelle totale fractionnée : 6 x 2 Gy en 3 jours (2 séances par jour).
  - (HBI) : Irradiation hémicorporelle.

BILAN.

8 patients ont été inclus dans l'étude : 2 (STBI), 2 (HTBI), 3 (FTBI) et 1 (HBI) En raison du délai nécessaire pour certains dosages, les résultats complets ne sont disponibles que pour 2 d'entre eux. Lors de cette première étape, l'étude biochimique a été limitée à : ACTH, cortisol, adrénaline, noradrénaline et amylase. Les prélèvements sont effectués pour les études hématologiques et biochimiques avant l'irradiation (2 à 3 prélèvements), pendant (2 à 3 prélèvements selon le type d'irradiation) et après, au moins pendant 3 jours.

IV. Objectives for the next reporting period:

Poursuite de l'étude des patients (environ 20 en 88) selon les modalités précisées en 1987.

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

Dr RAFFOUX - Laboratoire de greffe de moëlle France-Transplant.
Hopital St Louis. PARIS

Dr DOLOY - Laboratoire de Physiopathologie Expérimentale
Centre d'Etudes Nucléaires - FONTENAY - aux-ROSES

VI. Publications:

- - - - - - - - - - -

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-C-066-D

Institut für Strahlenhygiene
des Bundesgesundheitsamtes
Ingolstädter Landstr. 1
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Head(s) of research team(s) [name(s) and address(es)]:

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Institut für Strahlenhygiene
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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. Butkowskyj-Walkiw

I. Objectives of the project:

The aim of this study is to develop sensitive methods for early diagnosis of irradiation injury. The following features have to be investigated:
- radiation-induced changes in lymphocyte populations;
- radiation-induced changes in the electrophoretic mobility of human erythrocytes;
- changes in lectin-binding of different blood components after irradiation;
- changes in α-amylase enzyme activities after radiation exposure of radiotherapy patients;

II. Objectives for the reporting period:

In an attempt to discover a new "biological dosimeter", 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:
- radiation-induced changes in the electrophoretic mobility of human erythrocytes;
- changes in lectin-binding of platelets, leucocytes and erythrocytes after irradiation;
- changes in α-amylase enzyme activities after radiation exposure of patients;

III. Progress achieved:

During the reporting period we published results obtained for both electrophoretic mobility changes (EPM) of erythrocytes (Butkowskyj-Walkim et al., 1987) and dose-dependent increase of serum amylase in radiotherapy patients (Hofmann et al., 1987). Much insight could be gained into the reason for increase of serum amylase and the connection between increase and irreversible destruction of the salivary glands. In order to quantify the change in the lectin binding capacity of leukocytes, erythrocytes, and platelets following irradiation, several techniques have been optimized.

Electrophoretic Mobility Changes

As described in the last report there is a radiation-induced membrane-charge-change of erythrocytes which causes a different electrophoretic mobility behaviour. To examine the applicability of these changes as a rapid biological dosimetry system we conducted experiments using both erythrocytes irradiated in vitro and in vivo (radiotherapy patients). Under the conditions reported an effect could be measured after treatment of the cells with 0.6 % potassiumpermanganate in the dose range of about 3 to 5 Gy. However, the results from in vitro and patient samples were contradictory: where a decrease could be measured on in vitro exposed erythrocytes, the patient's cells showed an increase. This might be due to treatment with potassium-permanganate. So we are now working on conditions suitable to quantify the change of charge without any fixation process.

Lectin-Binding

Although there exists a broad spectrum of radiation-induced biochemical changes, their applicability as biological indicators of radiation exposure is often restricted due to physiological repair-mechanisms and the variations of the determined parameters between individuals in the same range as the induced effects.
At least the second point seemed to have been coped with by the binding of lectins to the membranes of human blood cells: Kubasowa (1981) reported differences in the radiation sensitivity of platelets, leucocytes and erythrocytes with increasing lectin binding capacities for doses exeeding 0.1, 0.5, and 3 Gy, respectively. This means it would be possible to detect a radiation effect by determining the cell-types which show increased binding. The normal binding can be measured 48 h after irradiation due to repair.
The proposed system has been designed so as to bind tritium-labelled Concanavalin A (Con A) to the cell membrane and seperate the free Con A from that bound to cells just by several washings. However, with this procedure we were not able to correctly quantify the binding due to the loss of cell material and ligand. We therefore worked out three other methods in order to achieve better results:
1. In order to reduce the loss of cells we performed the binding assay on the top of a bovine serum albumine (BSA) cushion in a microfuge-tube, seperated the free Con A by pelleting the cells and used the tube as a scintillation-vial after removing the BSA-solution.
2. Doing the binding assay in a microtiter-plate a good seperation of free ligand from cells can be achieved by filtration of the assay on glassfiber-filter using a cell-harvester.

Although the loss of cells can be reduced sufficiently by these two methods, the results like with Kubasowa's method depend upon absolutely correct determination of cell counts and upon the purity of the cell-fractions.
3. Use of a fluorescein label instead of the tritium label permits to determine the binding capacity of each single cell by means of a flow cytometer. This technique offers several advantages: Primarily the result does not depend upon the number of cells, secondly one can analyse cell-solutions containing more than one cell type, since the volume and light scattering are measured for each individual cell. Moreover leukocytes can be subdivided into lymphocytes and monocytes.
With none of these methods was it possible to define dose-dependent limits of increased binding for the different blood cells. In most of the blood samples investigated after in vivo and in vitro irradiation we found radiation-induced changes of lectin binding capacities. The degree of sensitivity, however, differed between individuals and also disagreed between in vitro and in vivo experiments.

Serum Amylase

The most promising effect in our opinion is the radiation-induced increase of blood concentrations of the sugar hydrolysing enzyme α-amylase. Although it was possible to establish a dose-effect relationship for the sera of therapeutically irradiatied patients, the average 24 hours values showed deviations in a very large scale from near starting point activity up to about 80 times the middle of normal range. Since dose assessments on this basis might be difficult, we tried to find the biochemical reason for the deviations and looked for possibilities to secure feasible conclusions.
Physiologically the effect of serum amylase increase is connected with damage to the salivary glands. Recovery of the salivary functions seems to be possible only if there is no complete destruction of secretory acinar cells. However, if the degree of injury is so extensive, that there is no saliva flow anymore, such damage seems to be irreversible. The amount of serum amylase increase is correlated with the degree of radiation-induced destruction of the glands. This means both effects probably have the same origin. Since the degree of radiation induced destruction can be modified by inhibition or stimulation of saliva secretion, these factors also may influence serum amylase concentrations. In fact the large scale deviations of serum amylase increase seem to be due to very different secretory conditions of the glands during radiation exposure. For the purposes of biological dosimetry this means that exact dosimetric predictions are only possible in consideration of further parameters which either describe such secretory state or which more directly specify the conditions of radiation exposure.
Nevertheless, evaluation of our investigations also provides more exact statements if consideration is given to the amylase effect only. For instance the kinetic studies reveal a steep radiation-induced increase of blood amylase after about 6 hours and the enzyme activity maxima in most patients occurs already 18 hours post irradiation and not at 24 hours as has been believed until now. The decrease in blood amylase is characterized by an exponential function thus with the 18 hours' maximum and with amylase activities at the time of dropping activity values it may be possible to draw conclusions not only about the level of the maximum but also about the starting point activity. Thus the time of utilization of the amylase effect as a biochemical indicator may be extended to about three days.

IV. Objectives for the next reporting period:

- Electrophoretic mobility changes
- Lectin-binding
- Serum amylase
- Single strand damage on DNA

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

Dr. N. Willich
Radiologische Abteilung des Universitätsklinikums Großhadern (München)

VI. Publications:

T. Butkowskyj-Walkiw, A. Stamm, R. Hofmann, N. Willich, A. Spiegelberg, J. Stanek, D. Pufal, W. Bögl,
Changes in blood components as biochemical indicators for irradiation. 20th Annual Meeting of the European Society for Radiation Biology, Pisa September 1986, Abstracts in: Int. J. Radiat. Biol. 51, 907 (1987)

T. Butkowskyj-Walkiw, R. Hofmann, A. Stamm, W. Bögl,
Radiation induced changes in blood components as biochemical indicators. 18th FEBS Meeting, Ljubljana, Yugoslavia, 28.6.-3.7.1987, Abstracts, pp. 184

R. Hofmann, T. Butkowskyj-Walkiw, N. Willich, A. Stamm, W. Bögl,
Enzyme activities of amylase in human blood - an indicator for exposures to ionizing radiation? 8th International Congress of Radiation Research, Edinburgh, 19.-24.7.1987, Proceedings, Volume I, pp. 94

R. Hofmann, T. Butkowskyj-Walkiw, A. Stamm, N. Willich, W. Bögl,
Strahleninduzierte biochemische Effekte und ihre Benutzung für Zwecke der biologischen Dosimetrie. 21. Hauptversammlung der Gesellschaft Deutscher Chemiker, Berlin, 13.-18.9.1987, Kurzreferateband pp. 173

R. Hofmann, D. Pufal, N. Willich, R. Westhaus, W. Bögl,
Biologische Indikatoren zum Nachweis von Strahlenexpositionen. Serumamylaseanstieg nach Bestrahlung der Speicheldrüsen. Bericht des Instituts für Strahlenhygiene des Bundesgesundheitsamtes, ISH-Heft 111, Juni 1987

T. Butkowskyj-Walkiw, A. Spiegelberg, W. Bögl,
Biologische Indikatoren zum Nachweis von Strahlenexpositionen. Untersuchung strahleninduzierter Veränderungen der elektrophoretischen Mobilität menschlicher Erythrozyten. Bericht des Instituts für Strahlenhygiene des Bundesgesundheitsamtes, ISH-Heft 112, Juni 1987,
Schriftenreihe Reaktorsicherheit und Strahlenschutz des Bundesministers für Umwelt, Naturschutz und Reaktorsicherheit, BMU-1987-164

T. Butkowskyj-Walkiw, A. Spiegelberg, W. Bögl,
Veränderungen der elektrophoretischen Mobilität von Erythrozyten nach einer Strahlenexposition. Tätigkeitsbericht des Bundesgesundheitsamtes 1986, pp. 93-95, MMV Medizin Verlag, München

G. Schreiber, A. Spiegelberg, K.W. Bögl,
Untersuchungen zur Anwendbarkeit der Lektinkopplung an Zellen des Blutsystems als "biologisches Dosimeter" bei Strahlenexpositionen. Tätigkeitsbericht des Bundesgesundheitsamtes 1987, MMV Medizin Verlag, München

R. Hofmann, D. Pufal, W. Bögl,
Strahleninduzierter Enzymaktivitätsanstieg von α-Amylase im Blut. Tätigkeitsbericht des Bundesgesundheitsamtes 1987, MMV Medizin Verlag, München

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-C-069-B

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
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Head(s) of research team(s) [name(s) and address(es)]:

Dr. A. Léonard
Département de Radiobiologie
CEN/SCK
Boeretang 200
B - 2400 Mol

Telephone number: 014-31.18.01

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. Leonard

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:

Preliminary results suggested that, as in other cells, caffeine could counteract the G2-arrest observed in mouse zygotes after irradiation. For 1987, we intended to determine precisely the concentrations of caffeine needed to obtain such effect, as well as the exact time at which it acts during the cell cycle. We also intended to explore different possibilities by which caffeine could exert its action, at the biochemical level. This could enable us to precise the main biochemical events which are inhibited by X-irradiation and restored by caffeine. Studies on chromosome aberrations in blocked embryos were undertaken in 1986 and would be continued. In addition, cytogenetic studies would be performed in irradiated embryos which escaped the one-cell block after incubation with caffeine.

III. Progress achieved:

1. Methodology

- Animals, irradiation

BALB/c mice were used, as in previous experiments. In one experiment, cytogenetic analyses were performed on embryos from two hybrid mice F1 (female BALB/c x male C57BL). These embryos do not undergo G2-arrest with the doses of irradiation used. Females were superovulated, mated and whole-body irradiated with doses from 0.25 to 2.5 Gy, at either 16, 19, 20 or 22 hours after hCG injection (4 to 10 hours after superovulation and fertilization).

- Cultures and treatments

Embryos were harvested by flushing the oviducts between 21 and 28 hours post hCG with medium containing hyaluronidase. Embryos with a second polar body and two pronuclei were rinsed and incubated at 37° C in a humidified atmosphere of 5 % Co_2 in air. Treatments were performed by incubating the embryos in culture medium containing caffeine (1 or 2 mM or more), in the presence, or absence, of cycloheximide.

- Cytogenetic analysis

For normal metaphase studies, embryos were cultured between 25 and 40 hours post hCG in medium containing 100 ng/ml of colchicine. Effects of caffeine were studied on embryos incubated between 28 and 40 hours post hCG, with colchicine and caffeine. Embryos blocked at the one-cell stage after irradiation were placed in colchicine-containing medium between 47 and 62 hours post hCG, for cytogenetic analysis in delayed metaphases. Embryos were fixed according to the classical method of Tarkowski (1966), and stained with lactic orcein.

2. Results

Embryos were incubated, immediately after irradiation of the mothers, in medium containing various concentrations of caffeine. With 2 mM caffeine, the G2-arrest induced by X-rays was almost completely suppressed. 1 mM caffeine was not so effective, and higher concentrations were toxic. Even at the 2 mM concentration, most embryos which had been induced to cleave without delay died during subsequent stages, but a small proportion of them reached the blastocyst stage after 4 days of culture. Embryos were incubated in 2 mM caffeine during different periods following irradiation. Results showed that caffeine must be present during the period of normal first cleavage (i.e. after 30 hours post hCG) to suppress the G2-arrest. Irradiated embryos were incubated a few hours before division (31 hours post hCG) with caffeine and cycloheximide. Results indicated that the inhibition of protein synthesis by cycloheximide completely prevents the action of caffeine on blocked embryos. Cytogenetic studies were at first performed on embryos able to cleave at the right time, despite irradiation. In such embryos, the proportions of chromosome aberrations varied as a function of the time of irradiation, showing clear relations with the varying

rates of lethality occurring from the morula stage (in previous experiments, we showed that irradiated embryos cleaving without delay develop normally up to the morula stage, at which mortality occurs). On the other hand, embryos undergoing a G2-arrest and analyzed in late cleavage, showed more chromosome damage than those escaping it and similar results were obtained in embryos forced to cleave at the right time by caffeine.
In addition, the proportion of triploids was significantly higher among delayed embryos than among those cleaving on time, reaching as much as 15-20 % after irradiation with 1 Gy at 20 or 22 hours post hCG.

3. Discussion

Our results clearly indicate that caffeine provokes a drastic inhibition of mitotic delay in irradiated embryos. This demonstrates that the one cell block is, in part, regulated by similar mechanisms to those observed in classical G2-arrest in other irradiated cells. Moreover, the embryo is receptive to this action of caffeine only during a short period, corresponding to the time of normal division. The action of caffeine apparently requires protein synthesis. Our observations on embryos cleaving at the right time agree with the general assumption that delayed lethality in irradiated preimplanted embryos is essentially due to chromatin loss occuring during successive divisions subsequent to chromosome breakage. Our experiments on delayed embryos as well as on embryos forced to cleave by caffeine suggest a correlation between G2-arrest and the presence of chromosome aberrations. However, some elements suggest that chromosome aberrations are not the only cause of G2-arrest :

1. Irradiation soon after fertilization (16 hours post hCG) does not induce G2-arrest, although embryos irradiated at that time exhibit high levels of chromosome aberrations at metaphase.
2. Embryos from F1 hybrids are not susceptible to G2-arrest, although presenting high levels of chromosome aberrations, as BALB/c embryos irradiated at the same time, and which undergo a G2-arrest.
3. We have shown that G2-arrest in mouse zygotes is a maternal effect, which means that the primary target of X-rays involves some radiosensitive factor synthesized from components of the oocyte cytoplasmic reserves.
4. In the mouse, the embryonic genome is not transcribed before the mid two-cell stage and therefore, does not intervene in modifications occuring during the one-cell stage, as well as in the first mitosis.

Finally, our results showing that triploids are preferentially blocked suggest that the nuclei contain the target for the X-rays induced G2-arrest.

IV. Objectives for the next reporting period:

Our actual results suggest that caffeine could act by stimulating the synthesis of some protein(s) necessary for division. This will be further investigated by different experiments involving 1) incubation of irradiated embryos with caffeine and cycloheximide according to different schemes ; 2) comparative study of protein synthesis and phosphorylation in control embryos and irradiated embryos incubated or not with caffeine. We also intend to determine if there exists a threshold dose for the occurrence of X-rays induced G2-arrest. Thus, the occurence of a G2-arrest, as well as its length, will be studied after irradiation with extremely low doses of X-rays. Extensive cytogenetic investigations will also be performed on embryos given the lowest doses inducing G2-arrest, in order to further investigate the role of chromosome anomalies in this effect.

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

Prof. Jaylet, Lab. de Biologie Générale, Univ. Paul Sabatier, Toulouse (France)
Prof. Streffer, Institut für medizinische Strahlenphysik und Strahlenbiologie, Universitätsklinikum Essen, Essen (FRG).

VI. Publications:

1. Publications in scientific journals :

S. Grinfeld, J. Gilles, P. Jacquet and L. Baugnet-Mahieu : Late division kinetics in relation to modification of protein synthesis in mouse eggs blocked in the G2 phase after X-irradiation. Int. J. Radiat. Biol., 52, 77-86 (1987).

S. Grinfeld and P. Jacquet : Existence of an unusual radiation-induced G2-arrest in the mouse zygote of the BALB/c strain. Int. J. Radiat. Biol., 51, 353-363 (1987).

S. Grinfeld, J. Gilles, P. Jacquet and L. Baugnet-Mahieu : Modifications post-traductionnelles de certains polypeptides en relation avec la première mitose chez l'oeuf de souris BALB/c. C.R. Soc. Biol., 181, 46-51 (1987).

2. Communications :

S. Grinfeld, P. Jacquet, J. Gilles and L. Baugnet-Mahieu : One-cell mouse embryo : a model for studies on G2-delay. 8th Int. Congress Radiation Biology, Edinburgh, July 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-C-071-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)]:

Dr. J.R. Maisin
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 an in utero irradiation on the central nervous system

Head(s) of project:
J.R. MAISIN, G.B. GERBER, H. REYNERS

Scientific staff:
E. GIANFELICI de REYNERS, L. REGNIERS

I. Objectives of the project:

Our aim is to reveal and study the mechanisms of fetal radiosensitivity using a combined morphological and biochemical approach. We also intend to determine the dependence on dose and radiation quality using a spectrum of irradiation protocoles.

II. Objectives for the reporting period:

A. Morphological assays

1. Study of the effects of irradiation in rats irradiated with very low doses of X-rays (5, 10 and 15 cGy, 250 KV) or Neutrons (1, 2.5, 5, 10 and 15 cGy, 600 KeV) at day 15 post-conception by means of a quantitative light microscopical analysis of long term changes in the brain (white matter) of female adult (3 month old) rats.
2. Autoradiographic analysis of the radiobiological behaviour of the residual population of embryonary cells still present in the adult rat cerebral hemispheres.

B. Biochemical assays

Determination of biogenic amines and amino acids in brain was carried out by HPLC chromatography using electrochemical and fluorescent (OPD) detection respectively. The benzodiazepine receptors in brain were determined using tritiated flunitrazepam. The preliminary studies on the distribution of benzodiazepine receptors were carried out by labelling in vitro brain slices with tritiated flunitrazepam and determining the distribution by autoradiography.

III. Progress achieved:

A. Morphological studies

a) Radiosensitivity of fetal brain

About 50 pregnant primiparous Wistar rats of similar weight were irradiated at day 15 post conception with 250 KV X-ray doses ranging from 0 to 15 cGy. Only litters of 8-10 pups were used in the following studies ; these animals were fixed by aldehyde perfusion of the brain when 3 month old. A similar protocole was also used after 600 KeV neutron irradiation but pregnant mothers were irradiated under Hypnorm anesthesia. The neutron experiments were repeated twice although variability was very low between the 108 brains collected.

Linear dose-effect relationships were observed for brain weight (BRW) :

BRW after 250 KV X-rays = 1.389 gm - 0.004 x Dose in cGy.
(sample size = 60 female brains, fixed when 12 week old)
BRW after 2.5 MeV Ntrn = 1.437 gm - 0.012 Dose in cGy.
(21 fem. brains, 13 week old)
BRW after 600 KeV Ntrn. (1st trial) = 1.378 gm - 0.019 Dose in cGy.
(54 fem. brains, 12 week old)
(2d trial) = 1.383 gm = 0.017 Dose in cGy. (54 fem. brains, 12 weeks).

For the 600 KeV neutrons (2d trial), the effect remained significant down to the 1 cGy dose level. The RBE was found to be 5.2 for the BRW criterion; this is twice the value observed with the 2.5 MeV neutrons.

Morphometric analyses dealing with more specific criterions (Integrating the different areas of the cingulum bundle measured by digitizer at various positions along the main brain axis) are still in progress for the above material. Preliminary data from X-ray and 2.5 MeV neutron experiments do not reveal significant alterations after doses lower than 10 cGy.

b) Radiobiological behaviour of the residual embryonary cells of the adult rat brain

The search for the subtlest morphological abnormalities present in the adult rat brain after an intrauterine irradiation requires an accurate knowledge of the tissue involved and particularly of the normal situation. We have largely extended such preparative work during the last reporting period ; our attention focussed on different tissue criterions which appeared as radiobiologically important :

1. the persistance of different categories of embryonary cells in the normal adult brain was mapped by autoradiography and found to be highly correlated with the areas at major risk of late radionecrotic damage (ref. 1).
2. the radiosensitivity of the vascular tissue (ref. 2,3).

B. Biochemical studies

Benzodiazepine receptors were determined 1, 3 and 6 months after exposure to 1 Gy (250 KV X-rays) at day 10, 12, and 15 of pregnancy. A decrease with age was observed in frontal cortex for B max (total amount of receptors) and Kd (dissociation). Lower Kd values were found in irradiated animals at an age of 1 month. At a later age, the differences between normal and irradiated animals seemed to disappear. In the cerebellum, differences appeared to depend on whether the calculation was carried out by a Scatchard linearization or by non-linear regression ; this might indicate that more than one component with different binding characteristics is present in the very young animals.

IV. Objectives for the next reporting period:

a. Morphology
1. Effects of 0.6 MeV neutrons on White Matter development (Cingulum).
2. Effects of fetal irradiation on the 15 month old brain.
3. Radiosensitivity of the residual embryonary cells in adult brain.

b. Biochemistry
1. Biogenic amines after 0.6 MeV neutrons in utero.
2. Opiate and histamine receptors (preliminary studies)
3. Automatic image analysis of benzodiazepine receptor distribution.

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

H. Liskien from EURATOM - C.B.N.M. (Geel, Belgium) and F. Poortmans of CEN/SCK (Neutron Physics, Mol) are responsible for neutron production and dosimetry. G. Konermann (Freiburg University) will provide assessment for image analysis studies.

VI. Publications:

1. E.G. de Reyners, H. Reyners and J.R. Maisin : Spatial relationships between late damage after X-irradiation and the zones of gliogenesis in the adult rat brain. Int. J. Radiat. Biol. 51 (1987) 750.
2. J.W. Hopewell, W. Calvo, D. Campling, S. Lauk, H.S. Reinhold, H. Reyners, M. Rezvani and T.K. Yeung : The role of the vasculature in normal tissue response. in : Radiation Research. Proc. 8th Int. Congress Rad. Res. Edinburgh (vol. II), pp. 789-794. Eds. Taylor and Francis, London 1987.
3. J.W. Hopewell, W. Calvo, A. Keyeux, H.S. Reinhold and H. Reyners : The pathogenesis of late radiation damage to the CNS : a multidisciplinary study in the rat brain. Proc. 6th European Congress of Radiology. Lisboa 1987.
4. in press (Glia, 1988)
   H. Reyners, E. Gianfelici de Reyners and J.R. Maisin : Radiosensitivity of Glial Progenitor Cells in the Rat Brain.

Title of the project no.: 2

Early and late radiation damage to the hematopoietic and immune systems of newborn animals

Head(s) of project:

L. de SAINT-GEORGES

Scientific staff:

G.B. GERBER, R. HOOGHE, M. JANOWSKI, L. de SAINT-GEORGES

I. Objectives of the project:

The working hypothesis is that the radiosensitivity of the haematopoietic 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 stem cells during the first year of life after postnatal irradiation.
2. The effect of prenatal or early postnatal irradiation on the immune system and in particular on the production of antiarsonate antibodies and cross reactive idictypes.

III. Progress achieved:

a-1) X-ray irradiated BALB/c mice, neonates and adults, were compared to nonirradiated mice for both a single dose (3.1 Gy) and a fractionated dose (1.1 + 3 Gy). The reticulocyte response was determined after a 3 days low pressure stimulation (350 mbar) at different times post irradiation (14, 30 and 90 days). The results demonstrated that the reticulocyte reaction differs profoundly between adult and neonate mice. In adult mice, a typical behaviour, with immediate damage, (reticulocyte depletion) followed by a recovery phase is seen during the acute period and a single irradiation has a more long-lasting depressing effect than a fractionated one.
In neonate mice, irradiation (single and fractionated) results in an immediate increase of reticulocyte release into the blood stream. At later times, the single exposure seems to have a larger effect than a fractionated one, although, in contrast to the behaviour of adult mice, the values are higher and recovery is better after a single exposure whereas an increased reaction is maintained after fractionated dose.

This strong reticulocyte response in neonates could be explained by a preferential stimulation of erythropoiesis in irradiated neonates compared to irradiated adults. Indeed, earlier it had been observed that the ratio erythroid to granulocytic type of spleen colonies increases in irradiated neonate mice up to day 30.

Alternatively since stromal cells of neonates are more radiosensitive than those of adults, an indirect stimulating effect on hematopoietic stem cells via a damage of the stromal cells could better account for the opposite reticulocyte reaction between adult and neonate mice.

a-2) In order to study the long term fate of hematopoietic stem cells, irradiated neoneatal (day 6) and adult mice exposed to lethal doses were rescued with a bone marrow transplantation consisting of homologous bone marrow cells with (B.M.) chromosome marker (metacentric chromosome issued from Robertsonian translocation). The mice were sacrificed at different time after B.M. cell injection and tested for the reappearance of the original host cells. This information should help to understand long term damage of the hematopoietic system as well as the possible relationship to leukemia development during the first year of life. The results are still being analysed.

b. In order to study the long-term effects of irradiation of the developing animal on the immune system, we have irradiated Wistar rats or A/J mice (0.25 - 2 Gy, X-rays, single exposure) at given times after conception (5-20 days) or after birth (2-8 days). At the age of 3 months, rats were immunized with a dinitrophenyl (DNP) derivative, either DNP-ovalbumin (a T-dependent antigen) or DNP-HES (T-independent). Mice were immunized with arsonate (Ars)-derivatized keyhole limpet haemocyanin.
The rat study has included so far the measurement of immunoglobulin levels of different classes and the levels of DNP-specific antibodies of each of these classes. No effect of irradiation could be disclosed on either total immunoglobulin or specific antibody levels after immunization with T-dependent or T-independent antigens.

In mice, the study was limited to a single dose of 0.5 Gy. In addition to levels of anti-Ars antibodies, Ars-related cross-reactive (recurrent) idiotypes of the A/J strain (CRI-A) were also measured with the idea that such a subpopulation of antibodies could be a sensitive indicator of damage consecutive to irradiation. Significant effects of irradiation were :

- compared to non-irradiated controls, the mice that had been irradiated at day 5 of gestation had reduced levels of anti-Ars and CRI-A ;
- mice irradiated between day 12 and 15 of gestation had reduced titers of anti-Ars in the primary response and increased levels after boosting ;
- in mice irradiated 18-20 days after conception, the level of CRI-A was often much higher than the level of anti-Ars, indicating that some specificity of the immune response was lost after irradiation.

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:

1. In order to confirm the different radiosensitivities between neonate and adult hemopoietic bone marrows, the reticulocyte response, in the same experimental conditions, will be evaluated in the C57BL mice strain which was found more sensitive to the low pressure stimulation and in the rats which do not show a in vitro preferential stimulation of erythropoiesis as do the mice.
2. Cell metaphasis from bone marrow transplantation experiments will be further analysed.
3. Idiotypic studies will be done on rat sera. After irradiation, we will also monitor bood and peritoneum cell populations.

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)

VI. Publications:

Topalova S., Hooghe R.J., Vander Plaetse F., Maisin J.R.
Effect of radioprotective glycans on the arrest of blood-borne lymphoma cells. Immunology Letters 15 (1987) 297-300.

Hooghe R.J., Maisin J.R., Vander Plaetse F., Urbain J., Urbain-Vansanten G.
The effect of prenatal and early postnatal irradiation on the production of antiarsonate antibodies and the cross reactive iodiotypes. Int. J. Radiat. Biol., in press.

de Saint-Georges L., Gerber G.B., Van Gorp U.
Capacity of the irradiated haematopoietic system of the neonate and adult mice to adapt to an increased demand. 8th International Congress of Radiation Research, Edinburgh, Scotland, 19-24 July 1987.

de Saint-Georges L., G.B. Gerber, Van Gorp U.
Capacity of the irradiated haematopoietic system of the neonate and adult mice to adapt to an increased demand. in : Stem cells in bone marrow after contamination with osteotropic radionucleides - EULEP Workshop, Antwerp (Belgium), 29-30 September 1987.

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:
A. LEONARD, M. JANOWSKI, L. de SAINT-GEORGES, M. LAMBIET-COLLIER, G. MATTELIN

I. Objectives of the project:

1. to study the effects of fractionation and radiation quality on induction and promotion of cancer in 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 groups 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
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.

III. Progress achieved:

I. Effect of fractionation and radiation quality on induction and promotion of cancer in mouse liver

1. Methodology

Three months old C57BL male mice were irradiated locally on the upper part of the abdomen with increasing doses of X-rays. Certain groups of mice received a single subcutaneous injection of 0.15 ml of 40 % CCl_4 in sesame oil either 69 h before X-ray exposure or at 3 months post irradiation. Control non-irradiated mice received a single injection of CCl_4. All CCl_4 injections and all X-ray exposures were performed between 9 and 11 a.m.

2. Results

The following groups of mice were treated :

- two types of controls : one receiving X-ray treatment and the other CCl_4 injection
  1. The irradiated control mice were subdivided in 5 equal subgroups and exposed to 0.5, 1, 2, 4 or 6 Gy
  2. Mice receiving subcutaneously 0.1 ml of a solution of 40 % CCl_4
- two types of treatment
  1. X-irradiation following CCl_4 treatment : 300 mice were divided in 6 equal subgroups treated with 0.1 ml CCl_4 and exposed 69 h later to 0.5, 1, 2, 4 or 6 Gy
  2. X-irradiation preceeding CCl_4 treatment : 300 mice 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.

The experiments are in progress and some preliminary results are available : About 50 % of the controls and the treated mice were dead on 1.1.1988. In both controls and treated group, the most important causes of death were leukemia (thymoma and non thymic lymphoma) and cancer of the liver (adenocarcinomas and sarcomas). No statistical significant differences were observed between controls and treated mice for the incidence of leukemia. The percentage of adenocarcinomas and sarcomas of the liver was significantly increased in the treated mice : X-irradiation following CCl_4 treatment and X-irradiation preceeding CCl_4 treatment. No difference seems to be observed between the two treated groups.

Conclusions

The results obtained so far seem to demonstrate that a combined treatment of a promotor given before or after an X-ray exposure seems to increase the incidence of liver cancer in C57BL mice. The results obtained for the group of mice promoted with CCl_4 3 months after irradiation seem to show that no time lack is needed for promotion.

II. 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. A publication was sent to Radiation Research and is now in press.

IV. Objectives for the next reporting period:

1. to carry on the analysis of the final results of mice exposed to X-irradiation following or preceeding CCl_4 treatment
2. to irradiate groups of mice with 0.5 MeV neutrons preceeding or following CCl_4 treatment for long term survival and cancer incidence.

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

BCMN, B-2440 Geel (neutron irradiation)

VI. Publications:

J.R. Maisin : Life shortening and disease incidence in C57BL mice after single and fractionated gamma and high-energy neutron exposure. Radiation Research, in press.

J.R. Maisin : Acute radiation syndromes in man. in press.

J.R. Maisin : Life shortening and causes of death in experimental animals following whole-body exposure to ionizing radiation. in press.

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, 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 diethylnitrasomine (DEN).

II. Objectives for the reporting period:

To study to which extent small doses of X-irradiation could potentiate the effects of diethylnitrosamine (DEN).

III. Progress achieved:

1. Methodology

Taking as a basis our preliminary data (cf progress report 1986) we have started a large scale experiment. A total of 2170 mice was distributed into the following 4 groups :

1. Mice treated with diethylnitrosamine (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 X-ray alone.
   Treatment at an age of 7, 14 and 21 days of age with a single dose of 0.5, 1 or 3 Gy of X-rays.
3. Mice treated with DEN + X-rays.
   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 0.5, 1 or 3 Gy of X-rays 7 days later.
4. Mice treated with X-rays + DEN.
   Treatment at an age of 7 days with a single dose of 0.5, 1 or 3 Gy of X-rays followed by 0.3125, 0.625, 1.25 or 2.5 µg/g of DEN 7 days later.

Ten mice of each group were killed at 10 weeks interval during a period of 70 weeks, starting at 10 weeks following carcinogenic treatment.

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. Five µm thick sections were cut 200 µm apart and stained with haematoxylin and eosin or with PAS. Four types of focal and nodular lesions were distinguished and recorded ; basophilic 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 (KONTRON) image analyser. This computer was connected 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.

2. Results

Preliminary experiments

Our preliminary data had shown that combined treatment with 0.3125 or 5 µg/g DEN and 0.95 or 2.85 Gy was most effective in increasing the number of hepatic nodules when the X-rays were given before the administration of DEN (table I). On the contrary, when X-rays were given after the administration of 0.3125 µg/g DEN the effect was less than expected from the sum of the action of the two agents (table I).

Large scale experiments

It is still too early to draw any conclusion from the first results obtained in the frame of the large scale experiment.

The progress achieved in the different groups of mice is as follows :

- Group I
All the mice of this group were treated with DEN.
Ten mice of each group were killed 10 to 50 weeks after treatment.

- Group II
All the mice of the group were irradiated.
Ten mice of each group were killed 10 to 30 weeks after treatment.

- Group III
All the mice of the group were treated with LEN and irradiated.
Ten mice of each group were killed 10 to 40 weeks after treatment.

- Group IV
The mice of this group were irradiated and treated with DEN.
Ten mice of each group were killed 10 to 30 weeks after treatment.

For all the four groups, the observations and the analysis of the results are in progress.

Table I : Number of microsccpic liver nodules and of hepatocarcinomas in mice killed at 64 or 78 weeks of age.

Treatments and number of mice		Number of microscopic nodules	Number of hepatocarcinomas
1. Controls	(10)	0,0,0,0,0 0,0,0,0,0	0,0,0,0,0 0,0,0,0,0
2. DEN 5 µg/g	(4)	5,6,31,3	5,6,5,3
3. DEN 1.25 µg/g	(10)	12,13,14,4,11 0,9,13,9,11	8,5,12,2,1 0,0,6,4,4
4. DEN 0.625 µg/g	(10)	0,2,0,0,2 0,2,0,5,1	0,1,0,1,0 0,2,0,0,0
5. DEN 0.3125 µg/g	(10)	4,3,0,1,0 2,1,2,1,2	1,1,0,1,0 1,1,1,0,2
6. 0.95 Gy + DEN 5µg/g	(10)	18,32,18,32,25 23,22,32,28,39	6,5,1,2,3 0,6,7,9,3
7. 2.85 Gy + DEN 5µg/g	(10)	35,24,12,10,3 6,8,1,13,18	6,1,4,0,0 0,5,0,6,1
8. DEN 5µg/g + 0.95 Gy	(10)	18,19,8,21,23 36,18,4,16,25	2,1,0,0,2 5,5,3,8,5
9. DEN 5µg/g + 2.85 Gy	(10)	5,12,21,23,17 31,14,17,13,21	5,6,2,6,3 10,6,1,5,1
10. 0.95 Gy + DEN 0.3125 mg/g	(10)	29,10,5,7,5 14,19,14,16,14	1,0,0,2,1 2,0,0,0,0
11. DEN 0.3125 mg/g + 0.95 Gy	(10)	0,0,0,0,0 0,0,0,0,0	0,0,0,0,0 0,0,0,0,0

Significant statistical differences for the number of microscopic liver nodules were observed in particular between the following groups : 2 and 6 ; 5 and 10 ; 5 and 11 ; 6 and 7 and 10 and 11 and for the number of hepatocarcinomas between the groups 5 and 11.

IV. Objectives for the next reporting period:

1. Most of the observations and the analysis of the results of the large scale experiment will be completed.
2. Groups of mice will be exposed to 0.5 MEV (0.1 ; 0.25 or 0.5 Gy of neutrons) preceeding or following DEN treatment to study long term survival and cancer incidence in the liver.

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, L. de Saint-Georges, M. Janowski, M. Lambiet-Collier and G. Mattelin : Effect of X-rays alone or combined with diethylnitrosamine on cancer induction in mouse liver. Int. J. Radiat. Biol. 51 (1987) 149-157.

J.R. Maisin : Protection against ionizing radiation by combinations of radioprotectors. Pharmacology and Therapeutics (1987) 53-55.

J.R. Maisin, S. Topalova, A. Kondi-Tamba, G. Mattelin : Radioprotection by polysaccharides. Pharmacology and Therapeutics (1987) 69-70.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-C-073-F

Commissariat à l'Energie
Atomique, CEA
Département de Protection Sanitaire
B.P. n° 6
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Telephone number: 1-654.85.85

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
D. GRUNWALD
(B. CHAPUT was absent from the laboratory for the year 1987)

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 objectives of the project are to evaluate the potentiality of flow cytometry towards 3 main goals: heterogenous chromosomal aberration detection on flow karyotype, 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:

Following the results obtained in 1986, the objectives were directed mainly on to only 2 of the initial 3 goals of the project.

I.FLOW CYTOMETRIC DETECTION OF RADIO-INDUCED CHROMOSOMAL DAMAGE : Quantification of flow karyotype parameters susceptible to variation after irradiation. Dose-effect relationship after *in vitro* irradiation of *Macaca fascicularis* monkey blood. Computer program for univariate flow karyotype analysis of different species.

II.CENTROMERIC DETECTION WITH FLUORESCENT ANTIBODIES : Ability of antikinetochore antibodies to detect dicentric chromosomes in irradiated cell culture. Suspension chromosomal labelling problems to solve: origin of the important FITC-background, selective loss of small chromosomes, quantification of FITC molecules per centromeric region, definition of the dicentric area on histograms and cytograms.

III. Progress achieved:

I - FLOW CYTOMETRIC DETECTION OF RADIO-INDUCED CHROMOSOMAL DAMAGE

1 Methodology

The lymphocyte isolation and culture, the chromosomal preparation for flow analysis have been described in previous reports. Uni- and bivariate flow karyotypes were obtained with a non-modified dual-beam flow sorter ATC 3000 (Odam-Bruker, Wissembourg, France) which allows both spatial and temporal resolution of the different fluorescences.

Chromosome suspension stained with ethidium bromide gave rise to flow karyotypes correlated with the DNA content of chromosomes, whereas the double stain Hoechst 33258/Chromomycin A3 gave access to the AT/GC contents of each chromosome.

Monkey whole blood irradiations were performed at 37°C with a 60Co source (dose rate: 0.5 Gy/min) and lymphocytes were isolated 1 H after irradiation and cultivated for 72 H.

2 Results

The smallest chromosome of Macaca fascicularis was well separated from the background of cellular debris in a monovariate flow karyotype. It was thus possible to define a value of the background (BF) in this area normally free of chromosomes in relation to the number of analysed chromosomes (fig 1a). 4 irradiation experiments on blood of 2 individuals are shown in fig 1b. Each experiment as well as the mean of the experiments showed a dose-effect relationship. However, if one wants to determine the value of the received dose from an isolated BF value through the mean standard curve, one can easily see some discrepancy in the results. This variability has certainly several causes: variability among the individuals and variability of the history of each individual (opposed to the stability of an in vitro cell culture), but certainly also the extreme dependance of the test on the way chromosome suspensions are prepared.

3 Discussion

These results, from blood isolated from two different individuals, are not very optimistic about the possibility of obtaining biological dosimetry when using such a method, contrary to the conclusions of the literature. But, most of the published data have been obtained on standardized in vitro cell cultures which are quite different from in vivo experiments performed here. However this method remains of some value for the detection of clastogenic agents in in vitro genetic toxicology tests.

II - CENTROMERIC DETECTION WITH FLUORESCENT ANTIBODIES

1 Methodology

Two human lymphoblastoid cell lines ICB 100 and 101 were used. Sera from patients with the CREST syndrome of scleroderma were the source of antikinetochore antibodies. Purified IgG were also used. The second FITC-labelled antibodies were anti-human IgG. All labelling was made on chromosomal suspensions. The flow cytometric analyses were carried out with dual-laser configuration for the detection of Hoechst-bound DNA fluorescence and the FITC-bound centromere fluorescence. Controls were performed either without first antibodies or with FITC-labelled serum albumin.

2 Results

Thanks to the possibility of analysing FITC fluorescence of the chromosome only (gate on fig 2a) it was possible to detect the binding of antikinetochore antibodies to the chromosomes. Although we found that the two centromeres of a dicentric chromosome obtained after cell culture irradiation are equally well labelled by antikinetochore sera, the existence of a tail on the FITC histogram (fig 2b) precluded the detection of these dicentrics by flow analysis. Control experiments showed that this parasitic fluorescence seemed to be centromere non specific but chromosome specific. The analysis by immunoblotting of the antigens recognized by the sera seemed to indicate the existence of some minor IgG able to recognize determinants other than the kinetochores.

3 Discussion

The presence of some chromosomes with higher level of FITC fluorescence may be due either to antibodies recognizing antigens other than the kinetochore or to kinetochores with different sizes varying with the chromosome lengths. The availability of monoclonal antibodies should allow to discreminate between these hypotheses and facilitate the flow cytometric detection of dicentric chromosomes.

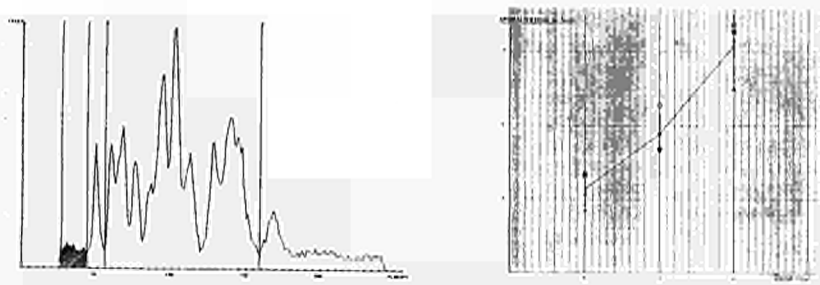

Fig 1 - Increase in the flow Karyotype background after irradiation (left: M. fascicularis flow Karyotype; right: dose-response relationship)

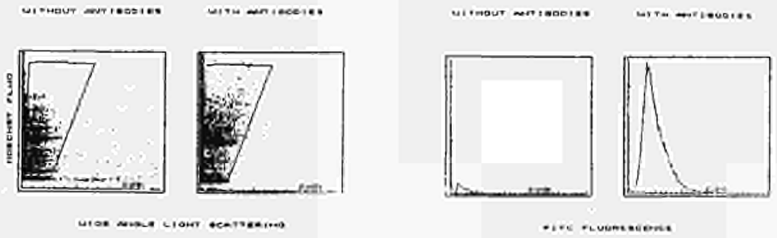

Fig 2 - Detection of FITC labelled kinetochore by flow cytometry (left: gate for chromosomal analysis; right: histograms of FITC fluorescence when antibodies are present or not)

IV. Objectives for the next reporting period:

Work in the next reporting period will concentrate mainly on the second goal of the project: labelling of chromosomal kinetochores with the aim of obtaining a better biological reagent. With the help of the Centre National de Transfusion Sanguine (Dr P. Rouger), human monoclonal antibodies against kinetochores will be searched. Monoclonal mouse antibodies supplied by Dr Earnshow (John Hopkins University, USA) will be also used.

Exchange of information and of samples will be also performed between members of the European Informal Group for Antikinetochore Labelling and Detection by Flow Cytometry formed during the Meeting of the Society for Analytical Cytology held in Cambridge (UK) in August 1987 (Drs J. Aten, Amsterdam, D. Green, Edinburgh, M. Nüsse, Frankfurt, and ourselves).

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

Dr JC Courvalin: Centre de Cytologie expérimentale. CNRS. 67, rue M. Gunsbourg. F94200 IVRY.
Drs JC Gaucher and A. Seigneur: Département d'Electronique Industrielle et Nucléaire. Service d'Instrumentation pour la Recherche. CEN Saclay. BP4. F91191 GIF/YVETTE CEDEX.
Dr MT Doloy: DPS. SPE. Laboratoire de Physiopathologie Expérimentale. même adresse.

VI. Publications:

* GRUNWALD D. (1987)
"Application de la cytogénétique en flux à deux grands mammifères: le porc (Sus scrofa domesticus) et le singe Cynomolgus (Macaca fascicularis)"
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* DELATTRE O., GRUNWALD M., BERNARD A., GRUNWALD D., THOMAS G., FRELAT G., AURIAS A. (1987)
"Recurrent t(11;22) breakpoint mapping by chromosome flow sorting and spot blot hybridization"
Human Genetics (in press)

* FRELAT G., THOMAS G., GRUNWALD D., AURIAS A. (1987)
"Flow karyotypes of human solid tumors: Studies of Ewing's sarcoma, peripheral neuroepithelioma and meningioma"
in: "Flow cytometry". A. Yen (ed.). CRC Press Inc. Boca Raton. USA. (commissioned)

* GRUNWALD D., FRELAT G., VAIMAN M. (1987)
"Animal flow cytogenetics"
in: "Flow cytometry". A. Yen (ed.). CRC Press Inc. Boca Raton. USA. (commissioned)

* PRUDHOMME J., GUILLY M.N., GRUNWALD D., SEIGNEUR A., COURVALIN J.C., FRELAT G. (1987)
"Flow cytometric detection of human chromosome kinetochores"
12th International Meeting of the Society for Analytical Cytology. Cambridge, Angleterre. 9-15 Aout 1987.
Cytometry, 8 (suppl. 1), 13.

* GRUNWALD M., GRUNWALD D., CHAPUT B., AURIAS A., FRELAT G. (1987)
"Detection of human chromosomal aberrations by uni- and bivariate flow analysis"
12th International Meeting of the Society for Analytical Cytology. Cambridge, Angleterre. 9-15 Aout 1987
Cytometry, 8 (supl. 1), 91.

* MENOU H., GRUNWALD D., CHAPUT B., FRELAT G. (1987)
"Semi-automated method for analysis of univariate flow karyotypes"
4ème Colloque Annuel de l'Association de Cytometrie en flux. Villefranche sur Mer. France. 29-30 Octobre 1987.
Biology of the Cell, 61 , 12a.

* MENOU H. (1987)
"Mise au point d'un programme informatique permettant d'analyser les courbes obtenues lors de l'analyse de chromosomes par cytometrie en flux"
Rapport de stage "Génie Biologique". 3ème semestre. Université de Technologie de Compiègne. Compiègne. Février 1987.
Rapport CEA.

* FRELAT G. (1987)
"Flow cytometry in radiation biology and radiation protection research: work at FAR"
Contractors meeting on the Application of Flow Cytometry Techniques in Radiation Biology and Radiation Protection Research (Prof. GB Gerber and Dr KH Chadwick, orgs.). Brussels. 21 May 1987.
Radiation Protection Program, CEC

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: B16-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
A Black
R J Talbot
J P Kellington

I. Objectives of the project:

a) To assess functional changes in pulmonary alveolar macrophages (PAM).

b) To study the kinetics of PAM recruitment into the alveolar spaces following exposure to airborne actinides.

c) To study nuclear aberrations in PAM and their possible role in predicting neoplastic transformations in pulmonary epithelial cells.

II. Objectives for the reporting period:

a) To continue work on metabolic activation of PAM at relatively low IADs of $^{239}PuO_2$.

b) To develop techniques for isolating Type II cells from disaggregated mouse lung.

c) To explore the use of flow cytometry in studying the effects of radiation on lung and, in particular, the phagocytic competence of PAM, the DNA content of disaggregated lung cells and the detection of nuclear aberrations.

III. Progress achieved:

Effect of $^{239}PuO_2$ on metabolic activation of alveolar macrophages

In the previous report we described how a histochemical technique had been used to demonstrate increased levels of lysosomal enzyme activity (ß-glucuronidase) in PAM from mice exposed to $^{239}PuO_2$. Although the results were based on a relatively high initial alevolar deposit (IAD) of ^{239}Pu (1100Bq), they did show that the most affected PAM were those that had engulfed particles of $^{239}PuO_2$. This year a similar study has been attempted using lower IADs (80 Bq and 240 Bq). In addition to the lungs receiving a lower radiation dose, fewer PAM would have contained particles, since the particle size distribution of the aerosol remained unchanged, In both groups, the average level of enzyme per cell was enhanced: those individual PAM containing ^{239}Pu showed by far the greatest response.

Induction of nuclear abnormalities in PAM

Most of our past work on the induction of abnormalities in PAM has involved high radiation doses. To extend these studies to lower doses, we have carried out a dose-response experiment using IADs of 20 to 600 Bq of $^{239}PuO_2$. The incidence, and total number, of PAM with a micronucleus (MiPAM), with 2 nuclei (BiPAM) and with both types of aberration (MiBiPAM) was measured at a single time-point (34 d). Evan at the lowest IAD there was a significant increase in the incidence of MiPAM. The yield of MiPAM/Bq IAD increased rapidly at low doses, as does tumour incidence. An account of this work is to be published shortly (Morgan et al, in preparation). It was found that the ^{239}Pu labelling indices of normal PAM and BiPAM were similar but fewer MiPAM were labelled.

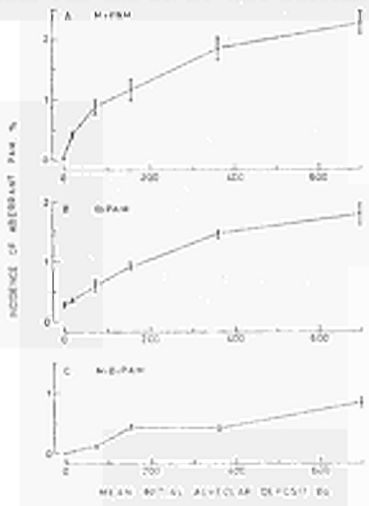

FIG 1. Incidences of MiPAM, BiPAM and MiBiPAM with increasing IAD of ^{239}Pu at 34d after exposure to $^{239}PuO_2$ (Mean ± SEM).

Isolation of epithelial cells from disaggregated mouse lung

This work is being carried out in collaboration with the Department of Biochemistry at Cardiff University. To isolate the epithelial cells, the lung is first lavaged with saline to remove PAM, and then selectively disaggregated by the instillation of proteases. The resulting cell suspensions are further purified by density gradient fractionation. Type II cells, which stain positive for alkaline phosphatase, have previously been obtained in high purity (>90%) from the rat lung. From the mouse, the purity of Type II cell suspensions obtained to date has been disappointingly low (~25%). Clara cell fractions have, however, been obtained with high purity (>80%).

In an attempt to improve on the purities of both the Clara and the Type II cells, we have investigated the use of fluorescence activated cell sorting (FACS). Early results are promising for selectively enhancing the yield of Type II cells (>70%). The use of FACS for sorting Clara cells appears less beneficial since they tend to clump in suspension: the flow cell cytometer requires a suspension of single cells to be able to give useful results.

Application of flow cytometry to studies of radiation effects on lung

i) Phagocytic competence of PAM

With FACS we are able to sort the free-cell population recovered from the mouse lung solely on the basis of forward- and side-scatter parameters. When 1 μm fluorescent beads are presented to PAM, in vivo or in vitro, the number of these particles taken up by a cell varies widely. With FACS we have been able to quantify and to sort pure (>98%) populations of PAM containing 0, 1, 2, 3, 4 or >4 particles per cell. It is hoped to determine if $^{239}PuO_2$-exposure inhibits the subsequent phagocytosis of these latex particles.

ii) Cell cycle analysis of PAM

In the previous report we described the use of Tritiated thymidine to study the origins of PAM with micronuclei. Now, with FACS, we are able to perform cell cycle analyses using the thymidine-analogue bromo-deoxyuridine (BrdU). This is injected 40 minutes before an animal is killed and the lungs are then lavaged. Cells in S-phase incorporate the BrdU into their DNA. By means of a fluorescent anti-BrdU antibody, we are able to quantify the proportion of PAM in S-phase without needing an autoradiographic step. This both enables many more cells to be scored and improves the reliability of the results.

IV. Objectives for the next reporting period:

a) To compare the phagocytic competence of PAM from control and $^{239}PuO_2$-exposed mice using fluorescent particles and flow cytometry.

b) To study the development of nuclear aberrations in PAM at lower doses than previously attempted (~10 Bq IAD). Also to continue to investigate methods of scoring micronuclei by flow cytometry.

c) To continue with attempts to isolate Type II cells and Clara cells from the mouse lung. If unsuccessful by May 1988 a decision will be made as to whether to continue with the mouse or to change to the rat. Attempts will then be made to quantify nuclear abnormalities in these epithelial cells, induced by exposure to $^{239}PuO_2$.

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

A EULEP Task Group on 'cellular indicators of early pulmonary changes' was set up in 1986. It includes outselves, the CEA at Bruyeres-le-Chatel, the Dept of Radiobiology at Bart's Medical College, and KFK at Karlsruhe. Also, we have been collaborating with Dr R Richards at Cardiff University on the disaggregation of mouse lung and isolation of Type II pneumocytes.

VI. Publications:

1. Moores S.R., Talbot R.J., Evans N. and Lambert B.E. (1986) Macrophage depletion of mouse lung following inhalation of $^{239}PuO_2$. Radiat. Res. 105, 387-404.

2. Moores S.R., Nicholls L.G., Talbot R.J. and Morgan A. (1987a) Long-term changes in the mouse lung during the development of pulmonary fibrosis induced by $^{239}PuO_2$. 1. Changes in the cells recovered by broncho-alveolar lavage. UKAEA Unclassified Report AERE-R 12377.

3. Moores S.R., Morgan A., Nicholls L.G., Roberts L. and Woodcock S. (1987b) Long-term changes in the mouse lung during the development of pulmonary fibrosis induced by $^{239}PuO_2$. 2. Changes in the enzyme content of pulmonary alveolar macrophages and in lavaged phospholipids. UKAEA Unclassified Report AERE-R 12831.

4. Morgan A., Moores S.R., Morris H., Nicholls L.G. and Talbot R.J. (1988) Induction of nuclear aberrations in mouse pulmonary alveolar macrophages following exposure to $^{239}PuO_2$. A dose-response study. UKAEA Unclassified Report (in preparation).

5. Talbot R.J., Knight D., Barnes P. and Black A. (1988) Enhanced macrophage-mediated clearance after inhalation of $^{239}PuO_2$ by mice. UKAEA Unclassified Report AERE-R 12997.

6. Walsh M. and Kellington J.P. (1988) Installation of a flow cytometry facility and some applications in radiobiology. UKAEA Unclassified Report AERE-R 12833.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 concerns 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 from functional membrane constitutions, influencing thus 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:

Early CNS lesion following to prenatal ENU-treatment can be seen first between postnatal day 12 and day 28 and consists of a local clustering of subependymal glial cells. This lesion only in rare instances will develop to a definite glioma. The mechanism of this regulative growth control is still unknown. Evidently, radiation-induced dystopic lesions of the germinative layer are producing a rather potent glioma-suppressing factor which displays its action even in CNS areas distant from the dystopia. This led to the following objectives: 1. Compilation of available data about growth-modulating mediators acting at the CNS during perinatal stages. 2. Experimental evaluation of the effects of one of these mediators in abnormal CNS development after ENU-treatment or X-irradiation in utero. 3. Histological assessment of the growth-modulating capacity of such a mediator upon the "early CNS lesion" following ENU-application in utero. We concentrate our studies on the influences of ß-endorphin within the above cited experimental set-ups. This neuropeptide was recently described to be responsible for radiogenic behavioural changes in mice and rats. As ß-endorphin was not available to us, we performed the experiments with use of its specific antagonist naltrexone.

III. Progress achieved:

1. Methodology

16 pregnant rats were divided randomly into 4 groups with 4 dams each. Group 1 remained untreated during the gestational period. Ethylnitrosourea (Sigma, Munich) was applied at a dose of either 50 mg/kg (group 3) or 80 mg/kg (group 4) by i.p. route in the morning of gestation days 14 and 18, respectively. The dams of group 2 were X-irradiated on gestation day 14 with help of an X-ray unit (200 kV, 10 mA, 0.3 mm copper plate filter, focus-target distance 40 cm, dose rate 0.01 Gy/sec.) with a total dose of 1.0 Gy.

Postnatal treatment: Pups from 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. During the weaning period 2 litters of each group remained untreated. Beginning on day 1, pups of the other 2 litters were given daily subcutaneous injections of naltrexone (Sigma, Munich) at a dose of 50 mg/kg until weaning on day 28. Solutions of naltrexone were prepared twice per week by dissolution in sterile PBS. The pups were weighed every 3 days and appropriate dosage and adjustments were made.

Evaluation: Animals were randomly selected for autopsy either on day 29 or day 30 of life. They were decapitated and brain, spleen and thymus were immediately removed for weighing. Subsequently these organs were fixed in 6% neutral-buffered formalin for additional histological studies.

2. Results

Daily injections of naltrexone had no effect on infant viability during the weaning period in all groups. Control animals with naltrexone treatment revealed significantly higher body weight than the postnatally untreated controls (Table 1). X-irradiation on gestation day 14 (group 2) significantly reduced the mean body weight of the offspring to 90.2 $\pm$ 7.1%, whereas both ENU-treatments remained without significant effects to the animals' weights.

Naltrexone treatment in groups 2-4 remained generally without significant effects on body weight (Table 1): In the X-ray pretreated animals a small increase of 3.9% contrasted with a weight reduction of 2.0% and 1.2% in the ENU-animals on days 14 and 18, respectively.

X-irradiation as well as ENU-treatment on g.d. 14 resulted in drastic brain weight reduction to 66.4 $\pm$ 3.3% and 78.4 $\pm$ 2.9% of the control weight, respectively. ENU on g.d. 18 reduced the brain weight only to 97.7 $\pm$ 5.0% of the control level. Naltrexone exerted significant effects on the brain weight in all groups. While the mean brain weight of the controls under the influence of naltrexone exceeded the weight of their untreated counterparts by 9.5 $\pm$ 0.1% (Table 1), there was an even greater recovery in the prenatally treated groups 2 and 3: X-irradiation plus naltrexone treatment led to a relative brain weight of 75.8% $\pm$ 3.7%, representing an increase of 14.1 $\pm$ 0.2% (Table 1) in relation to pups with X-irradiation alone.

Naltrexone applications to ENU-pretreated rats resulted in a brain weight of 88.5 ± 4.7% (group 3) and 106.7 ± 3.2% (group 4) of the control level. These data represent an amelioration of 12.9 ± 0.2% and 9.2 ± 0.1%, respectively. All these data were significantly different from controls at a level of 0.01 or lower.

3. Discussion

Our experiments revealed a significant stimulating effect of naltrexone on CNS development in normal rats. This effect is also obtained in prenatally injured offspring to a nearly equal or even higher rate. Apparently the effects of naltrexone on the newborn's brain does not vary with the extent of the embryotoxic injury. Rather, the response to naltrexone is increased in cases of disturbed brain development. By biochemical analyses it is further known that naltrexone is increasing the brain content of ornithine decarboxylase in 6-day-old rat pups. Recently we found this enzyme to be closely correlated with the appearance of the early histological lesions at the forebrain following prenatal ENU-application. Therefore our further interests are concentrated at the histological analysis of these preneoplastic brain lesions under the influence of naltrexone.

TABLE 1 Effects of daily naltrexone (NX) applications in the weaning period on body and brain weights from 28-day-old rats with either ENU-treatment or X-irradiation on gestation day (g.d.) 14 or 18

Prenatal treatment	♂ = Controls		X-irrad. (1 Gy)		ENU on g.d. 14		ENU on g.d. 18	
Postnatal treatment	♂	daily NX	♂	daily NX	♂	daily NX	♂	daily NX
1. Body weight (g)	54.9±0.7	61.3±0.5	49.4±0.8	51.4±0.6	54.2±0.5	53.1±0.5	54.8±0.5	54.2±0.6
relative change (%)		+ 11.7		+ 3.9		- 2.0		- 1.2
2. Brain weight (g)	1.43±0.02	1.56±0.03	0.95±0.01	1.08±0.02	1.12±0.02	1.26±0.01	1.39±0.03	1.52±0.03
relative change (%)		+ 9.5		+ 14.1		+ 12.9		+ 9.2

IV. Objectives for the next reporting period:

1. The histology of the ENU-induced early lesions under the influence of naltrexone (time-sequence studies).
2. Evaluation of a possible influence of naltrexone upon the formation of dystopic brain regions in response to prenatal X-irradiation.
3. Statistical evaluation of the time- and frequency pattern of gliomas following prenatal ENU-treatment alone or in combination with postnatal naltrexone applications.

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

VI. Publications:

Schmahl, W., Plendl, J., Reinöhl-Kompa, S.: Effects of naltrexone in postnatal rats on the recovery of disturbed brain and lymphatic tissues after X-irradiation or ethylnitrosourea treatment in utero. Res. Comm. Chem. Pathol. Pharmacol. 55, 89-99 (1987)

Weber, L.W.D., Schmahl, W.: The postnatal pattern of ornithine decarboxylase activity reveals a disparity of rat brain regeneration capacity after prenatal X-ray or 5-azacytidine treatment. Res. Comm. Chem. Pathol. Pharmacol. 56, 225-234 (1987)

Wiggenhauser, A., Schmahl, W.: Postnatal development and neoplastic disease pattern in NMRI-mice after combined treatment with ethylnitrosourea and X-irradiation on different days of the fetal period. Int. J. Radiat. Biol. 51, 1021-1029 (1987)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Universitätsklinikum Essen
Institut für Medizinische
Strahlenphysik und Strahlenbiologie
Hufelandstr. 55
D - 4300 Essen 1

Contract no.: BI6-C-077-D

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:

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 3 h p.c. (G1-phase) or 6 h p.c. (G2-phase). (h p.c. = hours post conceptionem)

2. Teratogenic effects after exposure of one cell embryos 3 h p.c. (G1-phase) to X-rays.

III. Progress achieved:

METHODOLOGY

1. Chromosome aberrations: One-cell mouse embryos were X-irradiated in vivo 3 or 6 h p.c. 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 zygotes 3 h p.c. and looking 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 is observed in comparison to the control. However, the different times of irradiation reveal different sensitivities: G1-zygotes (3 h p.c.) respond most sensitively, zygotes in S-phase (6 h p.c.) somewhat less, and 1 h p.c. zygotes are the least sensitive. This result corresponds qualitatively well to the survival rate in the teratogenesis experiments (1 h and 3 h p.c.; see Table 3). The number of aberrations

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 h p.c. are also quoted; these results have already been mentioned in the 1985 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
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
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

is markedly lower in the second mitosis after irradiation than in the first one and increases again in the third mitosis. This means that new aberrations must be produced rather late after irradiation.

The shift from chromosome type to chromatid type aberrations (Table 2) is to be expected. Chromatid type aberrations induced before the start of DNA-synthesis have been observed also in other systems and can be explained by various mechanisms.

Table 2: Ratio of chromosome to chromatid type aberrations in the first mitosis after irradiation (1- to 2-cell stage)

Time of irradiation (h p.c.)	Cell cycle phase	percentage of Chromosome type aberrations	Chromatid
Control		100	0
1	completion of 2nd meiotic division	83	17
3	G1-phase	58	42
6	S-phase	40	60

The number of malformed fetuses after irradiation either 1 h p.c. (completion of second meiotic division and start of decondensation of sperm nucleus) or 3 h p.c. (G1-phase) is very similar. The G1-zygotes, however, are slightly more sensitive with regard to survival. This is due to a higher rate of early resorptions, i.e. death shortly after implantation.

Table 3: Prenatal death and malformations after irradiation of 1-cell embryos. (The data of the 1 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)

* 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 irradiation in the 1-cell stage; time of irradiation: 9 h p.c. (early G2-phase) and 12 h p.c. (late G2-phase).

2. Teratogenic effects after exposure of 1-cell embryos 6 h p.c. (S-phase) and 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.
Streffer, C.: Risiko nach Strahlenexpositionen während der pränatalen Entwicklung des Menschen.
Strahlenschutz in Forschung und Praxis, Band XXVIII (1987) 34-47

2.
Weißenborn, U.; Streffer, C.: Chromosomal analysis of the first metaphases after X- and neutron-irradiation of one- and two-cell mouse embryos.
Poster on the occasion of the 8th International Congress of Radiation Research, Edinburgh, July 1987 (Abstract No. D41-1P, page 195 in Vol. 1 of the Proceedings of this Congress).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 tractions.

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. ir. L. Dorssers

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:

1. Adaptation of the serum free culture system for in vitro multiplication of stem cells established for murine bone marrow to conditions allowing primate stem cell multiplication:
   a. identification of the (human) hemopoietic growth factor(s) stimulating stem cell proliferation;
   b. comparison of the hormonal requirements in the cultures between murine and primate bone marrow.
2. Development of a method to purify primate stem cells.

III. Progress achieved:

Earlier, we reported the molecular cloning of the human hemopoietic growth factor interleukin-3 (IL-3). The biological activity of this hemopoietic growth factor has been characterized by in vitro studies and was found to have a similar range of activity on human bone marrow cells as murine IL-3 on mouse bone marrow cells. It stimulates proliferation of pluri- or multipotential hemopoietic stem cells, which then give rise to a wide variety of committed progenitor cells. Also in the human system, IL-1 potentiated the action of IL-3. The response of monkey bone marrow cells in vitro to stimulation with human IL-3 was considerably less than of comparable human cell suspensions, although the response to another hemopoietic growth factor, human GM-CSF, was extremely similar for bone marrow cells of these two species. The approaches to sort out this phenomenon include cell separation and stem cell purification experiments in search for accessory cells influencing the IL-3 response, as well as the molecular cloning of rhesus monkey IL-3. These experiments are being envisaged for 1988.

The development of serum free cultures for human and rhesus monkey hemopoietic progenitor cells has been completed by minor modifications of the murine serum free cultures reported from our laboratory.

Rhesus monkey stem cell purification has, in principle, been considerably facilitated by the availability of a monoclonal antibody against the human hemopoietic progenitor cell antigen 1 (HPCA1; CD34), which cross-reacts with rhesus monkey stem cells and progenitor cells (collaboration with Prof. R. Levinsky, Institute of Child Health, London, UK). It was demonstrated by in vivo autologous transplantation experiments that cells sorted for the presence of this antigen are capable of effective repopulation of lethally irradiated (12 Gy, given in two equal fractions separated by 24 hours) rhesus monkeys. Currently, scale expansion of stem cell purification, based on binding of the cells to immunomagnetic beads is being attempted in combination with the purification methods reported earlier.

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.
Department of Radiobiology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

VI. Publications:

Included in the publication list of project 2.

Title of the project no.:

2. Nonlethal 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, characterization and production of a lymphokine that suppresses the action of T-lymphocytes.

II. Objectives for the reporting period:

1. Selection of immunosuppressive monoclonal antibodies for studies in mice;
2. pilot studies with combinations of total body irradiation (TBI) and total lymph node irradiations (TLI) in mice;
3. development of a method to purify and produce the lymphokine that suppresses the action of T-lymphocytes.

III. Progress achieved:

Monoclonal antibodies to be used as adjuvant to TBI to achieve sufficient immunosuppression for sustained engraftment of T-lymphocyte depleted bone marrow grafts in mice will be obtained from the Department of Pathology, Cambridge University, UK (S.P. Cobbold et al., Nature 323, 1986, 164) as part of a collaborative study. The studies aim to extend the data on their immunosuppressive capacity in allogeneic mice. In connection with this part of the program, a take failure model has been developed in $F_1 \longrightarrow P$ mice (to eliminate any influence of immunosuppression from GvH-reactions) which allows a rapid and reliable screening of a variety of agents for immunosuppressive potency, which can be translated into a TBI-dose equivalent. The most immunosuppressive agents will then be tested in the same mouse model for possible synergistic or antagonistic actions and toxicity. It is intended to test the most promising regimens in mice for clinical feasibility in allogeneic rhesus monkey bone marrow transplantations with 4-log T-lymphocyte depleted grafts.

Sofar, the kinetics of the immunosuppressive actions of deoxycoformicin, goe 1734, cyclosporin-A and mitoxanthrone have been studied, and, on the basis of the results, these agents are currently being tested in the aforementioned mouse model.

A monoclonal Anti-LFA1 antibody has been identified that cross reacts with rhesus monkey cells. Anti-LFA1 will be used in rhesus monkeys as an adjuvant to TBI.

From the supernatant media of hybridoma cells two types of suppressor factors have been identified, a low molecular weight factor that interferes with the IL-2 response of T-cells and a high molecular weight factor that interferes with a very early event of T-lymphocyte activation. Only incubation of GvH-inducing cells with the latter factor, which on the basis of its preliminary biochemical properties probably is a single molecular species, will prevent GvH. Attempts to produce this factor at a larger scale will be continued.

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.
Department of Radiobiology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

VI. Publications:

L. Dorssers and A.M.E.A. Postmes.
A simplified, orientation-specific cDNA cloning strategy. Nucleic Acids Res. 15 (1987) 3629.

P.M. Hoogerbrugge, B.J.H.M. Poorthuis, A.H. Mulder, G. Wagemaker, L.J. Dooren, J.M.J.J. Vossen and D.W. van Bekkum.
Correction of lysosomal enzyme deficiency in various organs of β-glucuronidase-deficient mice by allogeneic bone marrow transplantation. Transplantation 43 (1987) 609-614.

S. Knaan-Shanzer and D.W. van Bekkum.
Soluble factors secreted by naturally occurring suppressor cells that interfere with in vivo graft-vs.-host disease and with T cell responsiveness in vitro. Eur. J. Immunol. 17 (1987) 827-834.

G. Wagemaker.
Selective multiplication of hematopoietic stem cells for bone marrow transplantation in mice and rhesus monkeys. Transplant. Proc. 19 (1987) 2721-2725.

E.P. Walma, H.M. Vriesendorp, C. Zurcher and D.W. van Bekkum.
Engraftment of stem-cell-enriched bone marrow fractions in MHC-identical dogs after fractionated total-body irradiation. Transplantation 43 (1987) 818-823.

R. Delwel, L. Dorssers, I. Touw, G. Wagemaker and B. Löwenberg.
Human recombinant multilineage colony stimulating factor (Interleukin-3): stimulator of acute myelocytic leukemia progenitor cells in vitro. Blood, 70 (1987) 333-336.

L. Dorssers, H. Burger, F. Bot, R. Delwel, A.H.M. Geurts van Kessel, B. Löwenberg and G. Wagemaker.
Characterization of a human multilineage-colony-stimulating factor cDNA clone identified by a conserved noncoding sequence in mouse interleukin-3. Gene 55 (1987) 115-124.

P.J. Heidt, H.A. Solleveld, A.H. Mulder and W.R. Gerritsen.
The influence of the donor microflora on graft-versus-host disease after allogeneic bone marrow transplantation in mice. Microecology and Therapy 16 (1986) 283-284.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-C-081-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. O. Vanderborght
Département de Radiobiologie
CEN/SCK
Boeretang 200
B - 2400 Mol

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 for bone tumor induction and myeloproliferative disorders. Early effects on stromal and hemopoietic stem cells of hemopoietic organs are studied in mice which received an injection of ^{241}Am citrate. 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 mice contaminated in utero with ^{241}Am and reared by a fostermother is continued. The goals are to evaluate increased risks for bone tumors or myeloproliferative disorders.
2 Long-term marrow cultures (LTC) in which the stromal cells maintain in vitro hemopoiesis are damaged in their capacity to produce hemopoietic stem cells (CFU-GM) after contamination of pregnant mice with ^{241}Am resulting in cumulative doses to the offsprings as low as 2 cGy (see last year's report). The origin of this damage is analysed.
3 The ^{241}Am concentration in the foetal skeleton after contamination of pregnant mice varied considerably between various bones : is this distribution correlated with the calcification and/or blood supply ?
4 Investigation of the extracellular matrix and celltypes in long-term cultures, derived from fetal and postnatal murine hemopoietic organs.

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 seperated and housed individually.
2. a. 3, 4, 6 and 15 weeks after in utero contamination, CFU-s, CFU-GM and CFU-f stem cell assays were performed from the bone marrow of the offsprings.
   b. An experiment was designed to test whether it was the stromal adherent layer of the LTC which was damaged in its capacity to sustain in vitro hemopoiesis. To compare the capacity of stromas from contaminated and non-contaminted offsprings, the stromal layers of respective LTC were irradiated (10 Gy X-irradiation) and recharged with fresh bone marrow from non-contaminated adult mice. Up to 3 weeks after recharging, CFU-GM numbers in the LTC were followed.
3. Ca and Fe measurements on ashed organs were performed using an I.C.A.P. emission spectrophotometer. The age of the mice from which the organs (femur, ribs, mandibula, calvarium and liver) were derived, ranges between 15 day-old fetus and 3 months.
4. We investigated liver and bone marrow LTC for the presence of extracellular matrix components (fibronectin, sulphated and non-sulphated glycosamino glycans (GAG) and celltypes of stromal and hemopoietic origin (alkaline phosphatase positive (AP+) fibroblast like cells, myeloperoxidase positive (MP+) cells, macrophages).
   AP+ cells, and MP+ cells were detected by means of enzyme histochemical assays. Macrophages could be distinguished through the ingestion of latex particles. Sulphated and non-sulphated GAG were differentiated with the high iron alcian blue histochemical staining method. Fibronectin was visualized with the immunochemical PAP method and determined quantitatively with an ELISA technique.

Results

1. This long-term experiment was continued and the injections of pregnant mice were finished. The experiment includes 157 pregnant mice which gave birth to 579 offsprings.
2. a. In situ 3, 4, 6 and 15 weeks after in utero contamination no differences were detected in the hemopoietic stem cell concentration (CFU-s, CFU-GM) and the stromal stem cell concentration (CFU-f) from non-contaminated control mice and ^{241}Am contaminated mice.
   b. The stromal adherent layers of LTC from control and ^{241}Am contaminated mice were equally effective in maintaining CFU-GM in vitro for 3 weeks.
3. The amount of ^{241}Am which initially deposits in a bone depends on the Ca concentration in the bone at that time.
   When the growth rate of a bone is high, the ^{241}Am concentration decreases rapidly.
   The fractional retention in the liver remains constant till one month of age. The subsequent decrease in ^{241}Am content occurs simultaneously with a decrease of the Fe content.
4. By choosing from each organ two ages, one with a high hemopoietic activity and one with a low hemopoietic activity, we tested whether the presence of the examined adherent layer components was correlated with the course of hemopoiesis.

All investigated components were present in liver and bone marrow LTC. Macrophages and GAG (sulphated and non-sulphated) show a positive correlation with hemopoietic stem cell number, MP+ cells which stand in our experimental conditions for granulocytes can only be detected in the adherent layer of adult bone marrow LTC although granulocytes are always present in the supernatant of the investigated LTC. Fibronectin and AP+ cells are not correlated with the hemopoietic activity but are inversely related with the proliferative capacity of the stromal stem cells from which the adherent layer was constructed. We suggest that fibronectin and AP+ cells are present in these cultures as a differentiation product of more mature stromal cells.

Discussion

1. This survival experiment will be continued.
2. It was demonstrated that radiation damage from ^{241}Am is manifest in long-term bone marrow cultures (LTC) from offsprings of contaminated mice reared by their own contaminated mother. At 6 weeks postcontamination, long-term bone marrow cultures derived from offsprings of contaminated mice were less able to support CFU-GM proliferation than control LTC from non-contaminated offsprings. It is not yet clear which component in the LTC (hemopoietic or stromal) is responsible for the radiation damage.
   In situ colony forming efficiency of hemopoietic stem cells (CFU-GM, CFU-S) and stromal stem cells (CFU-f) was not altered after the doses of ^{241}Am we used in our experiments. Thus differences in the concentration of the stem cells present at the onset of the cultures are not responsible for the observed radiation damage in LTC. Also, the stromas from ^{241}Am contaminated and non-contaminated mice after X-irradiation seemed equally effective in maintaining CFU-GM stem cells in vitro for 3 weeks as measured in a co-cultivation experiment with normal bone marrow. Different experimental set ups such as co-cultivation of both stromas with marrow from ^{241}Am contaminated mice has to be further studied.
3. The observed differences in ^{241}Am distribution cannot only be explained by changes in the Ca and/or Fe content. Further research must be done to elucidate this problem.
4. The capacity of LTC to maintain CFU-GM production is affected in cultures derived from ^{241}Am contaminated adult mice and in cultures from offsprings of ^{241}Am contaminated pregnant mice. To detect the radiosensitive components in these culture systems, characterization of the composing cell populations and the extracellular matrix components is performed. The obvious correlation between the presence of macrophages and GAG and the hemopoietic activity is the first step towards a further structural and functional analysis of these two components.

IV. Objectives for the next reporting period:

1. For the next year, survival data will be collected, post-mortem radiographs will be made from the skeleton and soft tissue histology will be performed.
2. Long-term bone marrow cultures derived from offsprings of ^{241}Am contaminated mice and from ^{241}Am contaminated adult mice are radiosensitive : a) does radiation damage persist : evaluation 1 year after contamination ; b) which are the radiation sensitive cell populations in these long term cultures ?
3. The assay for in vitro mineralization will be further characterized and its significance as a model for osteogenic differentiation of adult marrow cells will be evaluated.
4. The characterization of the stromal cell population (extracellular matrix components, production of growth factors, celltypes) will be continued.

V. Other research group(s) collaborating actively on this project [name(s) 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, S. Versele, G. Schoeters, O. Vanderborght
Stromal stem cells (CFU-f) in yolk sac, liver, spleen and bone marrow of pre- and postnatal mice.
British Journal of Hematology 66, 15-20 (1987).

S. Versele, R. Van Den Heuvel, G. Schoeters, O. Vanderborght
Proliferation activity of stromal stem cells (CFU-f) from hemopoietic organs of pre- and postnatal mice.
Radiation Research 111, 185-191 (1987).

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 (accepted July 1987), in press.

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 (accepted), in press.

G. Schoeters, R. Van Den Heuvel, C. Hurtgen, J. Colard
^{241}Am distribution in foetal haemopoietic organs of Balb/c mice.
Age related factors in radionuclide metabolism and dosimetry, ed. G.B. Gerber, H. Metivier, H. Smith, Martinus Nijhoff Publishers, Dordrecht, Nederland, pp. 193-200 (1987).

Van den Heuvel R., Schoeters G., Vanderborght O.
Radiosensitivity to ^{241}Am of bone marrow stromal cells in offspring of contaminated mice.
Age-related factors in radionuclide metabolism and dosimetry, ed. G.B. Gerber, H. Metivier, H. Smith, Martinus Nijhoff Publishers, Dordrecht, Nederland, pp. 201-208 (1987).

R. Van Den Heuvel, G. Schoeters, O. Vanderborght
Morphological changes in the stromal stem cell assay (CFU-f) derived from murine hemopoitic organs during gestation and postnatal life. (abstract).
European Journal of Cell Biology, Suppl. 20, Vol. 44, p. 54 (1987).

E. Mathieu, R. Van Den Heuvel, G. Schoeters, O. Vanderborght
Relation between hemopoietic activity and the presence of extracellular matrix and celltypes in the adhrent layer of long-term cultures from hemopoietic orgnas, during ageing (fetal and postnatal). (abstract).
European Journal of Cell Biology, Suppl. 20, Vol. 44, p. 45 (1987).

Schoeters G.E.R.
Adult mouse bone marrow cells mineralize in vitro.
Calcif. Tissue Int. V41, S2, pp 47 (1987) (abstract).

Eric Mathieu
Extracellular matrix and celltypes in bloodforming organs in vitro. Master's Degree Theses (1987). Biology Department, University of Antwerp.

Bernadette Rubbrecht
Correlation between Ca, Fe and ^{241}Am content in the liver and skeleton from pre- and postnatal mice. Industrial Engineer Degree Theses (1987), Denayer High-School, Mechelen.

Ingrid Leroy
In vitro mineralisation of bone marrow derived from the femur of adult mice.
Ind. Eng. Degree Theses (1987), Denayer High School, Mechelen.

III D

STRAHLENKARZINOGENESE

RADIATION CARCINOGENESIS

RADIOCANCEROGENESE

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-064-UK

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

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

Prof. G.E. Adams
Radiobiology Unit
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 to introduce male CBA/H mice into the main experiment and to begin to assess the results.

2. To develop further the software necessary for the storage, retrieval and analysis of experimental data from the mice.

3. To complete the experiment aiming to shorten the latent period for leukaemogenesis.

III. Progress achieved:

1. Myeloid leukaemia:osteosarcoma ratio

1.1 Methodology

Male 12-week old CBA/H mice were injected intraperitoneally either with 0.5cm^3 diluting solution (physiological saline containing 100µgcm^{-3} $^{2+}$Ca) or with 0.5cm^3 diluting solution containing ^{224}Ra.

The mice were allowed access to food and water ad libitum and were killed by exsanguination under chloroform anaesthesia either when they were judged to be able to live for no longer than one more day, were suffering or were showing specific signs e.g. splenomegaly with or without skin pallor. Smears were prepared from blood taken from a tail vein of those mice with suspected haemoblastoses and from the heart from each mouse which had been killed. These smears were stained with Leishmann's stain. Each mouse was radiographed and given a post-mortem examination. Samples of spleen, kidney, liver, sternum and lumbar vertebrae were taken routinely for examination under the microscope. Samples of other tissues were also taken if relevant abnormalities were seen. If leukaemia was suspected red and white blood cells were counted and aliquots of homogenized spleen were injected into female CBA/H mice. All tissue samples were embedded in wax and sections were stained with haematoxylin and eosin after prior decalcification in the case of bones.

Myeloid leukaemia was diagnosed from the morphological appearance of cells in blood smears and tissue sections in primary and passaged animals.

1.2 Results

A total of 1311 mice have now been introduced into the experiment; of these 397 have either died or been killed. Myeloid leukaemia has so far been diagnosed in 19 mice; the distribution of these animals in the injection groups is shown in table 1.

Table 1 Myeloid leukaemia:osteosarcoma ratio experiment
Status 30 December 1987

^{224}Ra injected (Bqg^{-1})	0	69	138	280	550
No. of mice injected	134	213	430	429	105
No. of mice dead	80	42	113	109	53
Myeloid leukaemia	0	2	7	7	3

1.3 Discussion

These interim results show highly significant yields of myeloid

leukaemia but do not yet show a significant trend in its incidence (taking competing causes of death into account) in those groups of animals given ^{224}Ra. This suggests that myeloid leukaemia is likely to be induced by amounts of ^{224}Ra smaller than $69 Bq g^{-1}$.

2. Shortening the latent period for the induction of myeloid leukaemia

2.1 Methodology

Twelve week old male CBA/H mice were injected with 16kBq ^{224}Ra by lateral tail vein. Four, fifty or one hundred days later marrow cells taken from the femur shaft or lumbar vertebrae of these mice were injected also via a lateral tail vein into 12 week old female CBA/H mice which had been lethally irradiated (10Gy X-rays) a short time previously. The recipient mice were given food and water ad libitum and were killed either when they appeared moribund or when a specific time period had elapsed. All mice surviving beyond 14 days were given a post-mortem examination and tissues were taken for sectioning as described in 1.1. Approximately equal numbers of mice were also injected with marrow cells taken from donors which had been injected with diluting solution only.

2.2 Results

All animals are now dead. The post-mortem examinations indicate that the major pathologies present at death are consistent with the effects of X-irradiation; myeloid leukaemia was not seen.

IV. Objectives for the next reporting period:

1. To continue to inject mice for the main experiment and to assess the results.
2. To develop further the software necessary for the storage, retrieval and analysis of experimental data from the mice.
3. To complete the experiment aiming to shorten the latent period for leukaemogenesis.

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

VI. Publications:

Humphreys, E.R., Green, D., Howells, G.R. and Thorne, M.C. (1982). Relationships between blood flow, bone structure and ^{239}Pu deposition in the mouse skeleton. Calcif. Tiss. Int. 34, 416-421.

Humphreys, E.R. and Loutit, J.F. (1980). Lesions in CBA mice from nanocurie amounts of ^{239}Pu. Int. J. Radiat. Biol. 37, 307-314.

Humphreys, E.R., Loutit, J.F., Major, I.R. and Stones, V.A. (1985). The induction by ^{224}Ra of myeloid leukaemia and osteosarcoma in male CBA mice. Int. J. Radiat. Biol. 47, 239-247.

Humphreys, E.R., Loutit, J.F. and Stones, V.A. (1985). The induction, by ^{239}Pu, of myeloid leukaemia and osteosarcoma in female CBA mice (Interim results). In 'Metals in bone' (Edited by N.D. Priest). MTP Press Limited, Lancaster, Boston, the Hague, Dordrecht, for the Commission of European Communities (1985) pp. 343-351.

Humphreys, E.R., Loutit, J.F. and Stones, V.A. (1987). The induction by ^{239}Pu of myeloid leukaemia and osteosarcoma in female CBA mice. Int. J. Radiat. Biol. 51, 331-339.

Humphreys, E.R. and Papworth, D.G. (1986). The dosimetry of ^{224}Ra in mouse bone. Int. J. Radiat. Biol. 50, 621-629.

Humphreys, E.R., Papworth, D.G. and Stones, V.A. (1984). ^{220}Rn retention in mouse bone. Radiat. Environ. Biophys. 23, 145-148.

Humphreys, E.R., Papworth, D.G. and Stones, V.A. (1986). The leukaemogenic dose from ^{224}Ra in male CBA mice. In: "The radiobiology of radium and Thorotrast" (Edited by Gössner, W., Gerber, G.B., Hagen, U. and Luz, A.) Strahlentherapie Supplement to volume 80 pp 83-87.

Humphreys, E.R., Robins, M.W. and Stones, V.A. (1985). Age related and ^{224}Ra-induced pathology in the teeth of CBA mice. Archs. oral Biol. 30, 55-64.

Humphreys, E.R. and Stones, V.A. (1980). Mixed ligand chelates and plutonium poisoning. Health Phys. 39, 103-105.

Schofield, R., Lord, B.I., Humphreys, E.R. and Stones, V.A. (1986). Effects of ^{239}Pu, on haemopoiesis. 1. Quantitative and qualitative changes in CFU-S in different regions of femur and vertebrae. Int. J. Radiat. Biol. 49, 1021-1029.

In Press January 1988

Humphreys, E.R. and Humm, J.
A Monte-Carlo approach to the microdosimetry of ^{224}Ra in murine compact and cancellous bone. Health Physics.

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 radionuclide ^{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 ^{224}Ra-induced myeloid leukaemias as they arise in irradiated CBA/H mice and to develop computer-assisted analysis systems.
2. To continue studies on clonal proliferation following in vitro α-particle irradiation and transplantation.
3. To initiate in vitro studies which attempt to relate induced chromosome 2 change with cellular differentiation or viral processes.
4. To continue studies on DNA structure and mRNA expression of chromosome 2 encoded genes.
5. To attempt to construct chromosome 2 specific DNA libraries from flow-sorted murine chromosomes.

III. Progress achieved:

1. Methodology

1.1 Karyotypic analyses: The karyotypes of acute myeloid leukaemias (AMLs) of derivative cell lines and of somatic cell hybrids were analysed using G-banding techniques. A total of 100 G-banded CBA splenocyte metaphases were prepared and analysed in detail in order to provide a data base for computer-assisted karyotypic analyses (in collaboration with Drs. J. Piper and D. Rutovitz).

1.2 In vitro cellular irradiation and transplantation: Initial karyotypic analyses of in vitro α-particle irradiated bone-marrow cells indicated considerable dose-inhomogeneity. Improvements in the irradiation technique were followed by a series of experiments (in collaboration with Drs. E. G. Wright and D. T. Goodhead) to determine the dose-effect for transplanted cell survival using spleen colony (CFU-s) assays.

1.3 Molecular analyses of genes in normal and leukaemic cells: High M.W. DNA and mRNA were extracted from cells using standard preparative techniques. Restriction-enzyme digested DNA and mRNA were electrophoresed on agarose gels, subjected to standard blotting techniques and hybridized to ^{32}P radiolabelled gene probes. Hybridizing nucleic acid species were detected by autoradiography.

2. Results

2.1 Karyotypic analyses: Analyses of radiation-induced AMLs has further strengthened the association between specific deletion/rearrangement of ch2 and this murine haemopoietic neoplasm (2,3). Of particular significance was the characterisation of a novel 2;11 translocation in one X-ray induced myelomonocytic leukaemia. Karyotypic analyses have also confirmed the presence of the ch2 rearrangement in AML derived cell lines obtained after infection of leukaemic cells with transforming recombinant retroviruses and also in panels of Chinese hamster x AML somatic cell hybrids obtained through polyethylene glycol-mediated cell fusion.

Development of computer-assisted karyotypic analysis is now nearing completion and the system should be operational during the next reporting period.

2.2 In vitro cellular irradiation and transplantation: Quantitative clonal survival techniques (CFU-s) for in vitro α-particle irradiated bone-marrow cells have provided a reliable measure of haemopoietic stem cell radiosensitivity; the D_o value for α-particles from Pu was $\sim$0.5 Gy. These

data are now being used in the design of experiments to quantify the induction f ch2-rearranged stem cell clones by α-particles with a view to comparing α-particle induction with that previously reported for X-rays (1)

2.3 Molecular analysis of genes in normal and leukaemic cells: In the previous rep rt we failed to sh w any restriction fragment polymorphism in the ch2 encoded genes ABL, SRC and B2M of murine AMLs. The preliminary con lusion that these genes were not inv lved directly in the characteristic ch2 rearrangements has been further strengthened by mRNA analyses which sh w no evidence of consistent change in AMLs and also by the failure to find any novel ABL proteins (1,2). In order to broaden our approach we have investigated the structure and function of haemopoietic growth fact r/receptor genes in AML. No obvious DNA or mRNA changes have been seen amongst 5 AMLs (including the t2;11 AML) in the genes encoding IL-3 (ch11), G-CSF, M-CSF, FMS or ERB (ch11). Preliminary studies do, however, indicate that rearrangement and inactivation of GM-CSF (ch11) may not be uncommon in AML.

3. Dicussion

Cytogenetic studies in this laboratory have clearly shown that specific rearrangement of ch2 is a characteristic feature of radiation-induced AML in the CBA mouse and that such rearrangements may represent radiation-induced initiating events in multipotential haemopoietic cells.([1]) Molecular studies have failed to directly implicate the ch2 encoded genes ABL, SRC and B2M in these rearrangements (1,2). Since normal and leukaemic haemopoiesis is subject to proliferation/differentiation control through the action of cell lineage-specific growth factors (CSFs) we have initiated an investigation of the structure and activity of the DNA sequences that specify the most well characterised CSFs, i.e. granulocyte(G)-CSF, macrophage(M)-CSF, granulocyte-macrophage(GM)-CSF and interleukin(IL)-3. While the GM-CSF gene rearrangement and inactivation seen in some AMLs may be significant, particularly in terms of leukaemic development, there is no evidence that they play a r le in radiation-induced initiation processes. Our cytogenetic data ([1]) strongly imply that target gene(s) for initiation are encoded on ch2. Recently, sequences encoding IL-1α and β have been assigned to murine ch2 (Immunogenetics, 26, 339, 1987) and their direct relevance t> AML is suggested by the constitutive expression of the β gene in the equivalent human ne plasm (Blood, 70, 1218, 1987). Amongst other properties IL-1 gr wth fact rs are thought to act on early haemopoietic

precursor cells and, albeit through circumstancial evidence, these genes now represent the favoured candidates for AML initiation in the CBA mouse.

IV. Objectives for the next 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 transplantation.
3. To continue studies on DNA structure and mRNA expression of proto-oncogene and growth factor gene sequences.

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.

Drs. E. G. Wright and D. T. Goodhead, MRC Radiobiology Unit, Chilton, Didcot, Oxon, U.K.

VI. Publications:

1. SILVER, A.R., BRECKON, G., MASSON, W.K., MALOWANY, D., COX, R. (1987). Studies on radiation myeloid leukaemogenesis in the mouse. Radiation Research: Proceedings of the 8th International Congress of Radiation Research, ed. E.M. Fielden et al. Vol. 2, 494-500. London: Taylor & Francis.

2. SILVER, A.R., MASSON, W.K., BRECKON, G., COX, R. (1988). Preliminary molecular studies on two chromosome 2 encoded genes, c-abl and B-2M in radiation-induced murine myeloid leukaemias. International Journal of Radiation Biology. In press.

3. BRECKON, G., SILVER, A.R. and COX, R. (1988). Consistent chrcmosome changes in radiation-induced murine leukaemias. Proceedings of the Kew Chromosome Conference III. In press.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

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

Contract no.: BI6-D-067-NL

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

Prof. Dr. G.W. Barendsen
Division for Health Research
Radiobiological Institute TNO
Lange Kleiweg, 151
NL - 2280 HV Rijswijk

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.: 1

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 effect 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:

The measurement of radiation induced chromosome damage by flowcytometry and its correlation with the induction of cell reproductive death, as described extensively in the previous annual report, depends on the optimization of flowkaryometry. Because of the small size of some of the chromosomes and the corresponding by weak fluorescence which has to be detected with good sensitivity accuracy and reproducibility, flowkaryometry requires biological preparation methods and physical instruments in which high intensity narrow laser beams illuminate chromomes of well defined morphology.

Especially in applications to human chromosomes these conditions are even more stringent than for the V-79 Chinese hamster cells which were employed in the earlier studies, because the smallest chromosomes of human cells are much smaller than those of Chinese hamster cells.

For investigations of DNA contents of individual chromomes and of changes in DNA contents after irradiations, it is important that the fluorescent signals and their measurements accurately reflect the DNA contents of the chromosomes. Investigations have been performed during the past year directed at improving the high resolution flowcytometer for chromosome analysis. In particular the detection system of the fluorescent light could be improved by changes in the electronic signal analysis. Using the peak fluorescence of chromosomes of Chinese hamster cells, the ratio of the DNA contents of the largest and the smallest chromosomes of NBCH-3 diploid CHinese Hamster cells was mreasured to the equal to 4.4. Using the integrated fluorescence the same ratio was assessed at 6.2. This difference was analyzed by detailed studies with different chromosome preparation techniques involving long (18 h) and short (2-4 hr) applications of colchicine or vinblastine. It could be shown that the preparation of the chromosomes had no influence on the relative fluorescence of the chromosomes of different lengths. The difference between the measurements using peak fluorescence and integrated fluorescence is most likely due to the fact that the long chromosomes are longer than the width of the laser beam. Consequently, the peak fluorescence is not a good measure of DNA content.

Analysis showed further that the results using the integrated fluorescence agreed better with data obtained in the Laboratory for Radiobiology in Amsterdam (Dr. Aten) and in the Life Sciences Division in Los Alamos USA (Dr. Wilder). For the same NBCH-3 cell line corresponding ratio's of fluorescence of the largest and smallest chromosomes of Chinese hamster cells were measured at 7.7 in Amsterdam and 6.7 in Los Alamos. It can be concluded that measurements of the fluorescence of chromosomes integrated over the time during which they pass through the narrow laser beam rather than the peak fluorescence provides the best measure of the DNA content, but the cause of remaining differences have yet to be elucidated.

Studies of reproductive death of cells cultured in vitro in past years have been performed with different cell lines and with neutrons and alpha particles of different energies. In an analysis of these data it was calculated that for cells which had attached to dishes and had spread out to a flat shape, the actual cross section of the cell nuclei perpendicular to the beam of alpha particles can vary for T-1 human cells used in older experiments as well as for C3H 10 T½ mouse cells and NBCH-3 Chi-

nese hamster cells from values of about 70 um^2 to values in excess of 300 um^2. Nevertheless, for irradiations with alpha particles using the track segment method the cross section for inactivation, derived from own data an published results did not vary greatly. For T-1 human cells the maximum cross section with LET values in excess of 100 keV/um was 35 um^2, while for V79 cells and C3H 10T½ cells values between 30 and 50 um^2 and for HF human diploid cells a value of about 60 um^2 have been published. It could be concluded that the probability of inactivation for an alpha particle at the most effective energy of about 2-3 MeV can be described by an effectiveness per unit particle track length through the cell nucleus of between which are all within a relatively narrow range of 0.05 to 0.08 per um. This would imply that if an alpha particle of this energy passes through a spherical cell with a nuclear volume of 500 um^3 and a diameter of about 10 um, the mean track length in the nucleus is 6.3 um and the probability by a single particle of inactivation could be as large as 0.5, while for a flat shaped cell with a thickness of 2 um this probability may be as low as 0.1. It can be concluded that no discrepancy exists between data published in the literature about the number of alpha particle tracks through the nuclei required to inactivate mammalian cells, because the observed differences can be ascribed to the time interval between plating and irradiation of cells, which is associated with attachment and spreading of cells on the bottom of culture dishes. In early experiments with T-1 human cells this time interval was only 4 hours and cells had not spread out at the time of irradiation, while in many later studies time intervals of 24 hrs between plating and irradiation were used. For an extrapolation to cells irradiated in the human body, e.g. lung cells irradiated by alpha particles from radon daugthers, a spherical shape of the nucleus is probably more relevant than a flattened shape.

IV. Objectives for the next reporting period:

Studies will be continued to obtain data and analysis 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, 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 of 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. Flowkaryometry has been employed to study changes in chromosomes associated with transformation. Detectable alterations in flowkaryograms 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 25 clones have now been analysed and in 12 clones chromosome structural or numerical changes were observed. A consistent pattern 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 can be 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 develoment 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.

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, Dr.Ir. B. Hogeweg

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 from 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 population 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 to 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 exposed to various regimes of radon, formaldehyde and acetaldehyde, respectively. As reported earlier, for the exposure of the rats to radon daughters, a "nose-only" inhalation chamber combined with recycling and reconditioning of the air in a CO_2 absorber will be optimalized.
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:

Studies have been contintued to optimalize the exposure arrangement of rats to radon and radon daughters at levels sufficient to induce lung cancer.
For the analysis of a possible interaction on lung tumorigenesis from the inhalation of radon combined with other toxic agents, as discussed and reported earlier, a radon daughter exposure level of 1000 to 2000 WLM would be optimal. For an exposure time of 6 h. and a total number of 10 exposures, it can be calculated that a concentraton of 3000 to 6000 WL is required. It can be estimated that, for a closed system, for these exposure levels about 0.7 to 1.4 MBq radium per dm^3 is required. In order to restrict the total amount of radium, a "nose-only" inhalation chamber with a volume of 6 dm^3 for the simultaneous exposure of 24 animals was constructed.

The system was completed and tested for the radon concentration at a number of flow rates. A water vapour-aerosol generator was installed in order to increase the probability of attachement of radon daughters to particles in the inhaled air. The efficiency of the radon mixture increases with increasing build up period, but this is associated with a low ventilation rate. However, due to the enhanced CO_2 concentration in the expired air, a low ventilation rate did result in hyperventilation of the animals. Measurements showed that for the constructed "nose-only" chamber an air flow of about 11 dm^3 per min is required in order to prevent this hyperventilation. This corresponds to a mean residence time of the radon in the chamber of about 0.5 min. Since the radon daughter concentration is strongly dependent on the residence time, the concentration measurement have been performed for the air flow of 11 dm^3 per min.
Radon concentrations expressed as working levels (WL) were measured as described in the previous annual report. The highest level of about 12000 WL at an RaA/RaC ratio of 0.57 was measured for conditions where no rats were present. For other flow rates WL levels were in the range of 4000-10000. However, when rats were placed in the exposure system the concentration decreased greatly to levels of 200-400 WL. It is evident that the inhalation and retention of Radon daughters in the rats greatly influenced the exposure conditions and caused decreases by factors of 10 to 20. Levels between 1000 and 2000 WL were aimed at for further studies.
In order to obtain insight in the amounts of radioactivity in different organs of these rats measurements were performed of gamma radiation emitted by Pb-214 and Bi-214. An example of these measurements is presented for lung. The activity decreases corresponding to the desintegration constant of Pb-214. Extrapolated values indicate that values of 1000-2000 WL are not yet obtained.
In order to attain higher exposure levels, collaboration is sought with the Institut de Protection et de Sureté Nationale, Fontenay-aux-Roses (Dr. Lafuma).

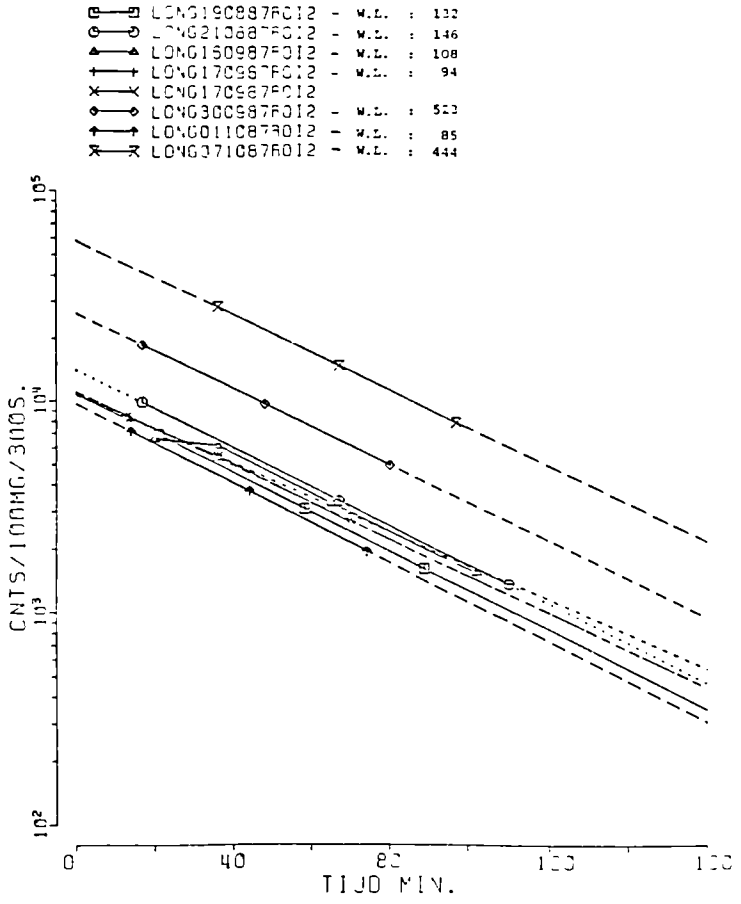

Figure 1. Radioactivity in lungs of exposed rats.

IV. Objectives for the next reporting period:

The plans for the coming year are:
a). finalizing of the construction of the exposure arrangement with re-circulation system;
b). measurements of the radon daughter concentration levels;
c). exposure of WAG/Rij rats to the two proposed radon daughter levels of 1000 and 2000 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-onderzoekprogramma naar het achtergrondniveau van de natuurlijkse straling in Nederland. Rapportnr. 3477, Radiobiologisch Instituut NTO, Rijswijk (1986).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: B16-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 the project no.:

RADIATION CARCINOGENESIS: FOLLOW-UP OF CANCER PATIENTS TREATED BY RADIOTHERAPY FOR THE APPEARANCE OF A SECOND INDEPENDENT TUMOUR.

Head(s) of project:

ALDO BECCIOLINI
ENRICO CELLAI

Scientific staff:
BALZI M.,CHIAVACCI A.,OLMI P.,PUPI A.,CASTAGNOLI A.,BOANINI P., SCUBLA E.,PORCIANI S.,LANINI A.,SANTONI R.,CIONINI L.,MUNGAI R. PONTICELLI P.,MUNGAI V.,CREMONINI D.,ZANIERI E.,MAURI P., MAGNOLFI A.,DE CRISTOFARO M.T.R.,BITI G.,CINTOLESI V.,PACINI P.

I. Objectives of the project:

Radiation carcinogenesis induced by low doses is an infrequent phenomenon and therefore difficult to quantify. However, in patients affected by tumours very high doses are administered in a restricted volume where generally the dose is so high as to destroy even the healthy cells. Normal cells with sublethal damage can undergo a process of transformation. During this exposure much lower doses are received by other parts of the body. The appearance of a second tumour could be a possible consequence of ionizing radiation treatment. It must be noted however that many other factors can contribute to the appearance of a second tumour, given the particular conditions (i.e. immunologic status etc.) of the neoplastic patient.

II. Objectives for the reporting period:

1) Analysis of follow-up and clinical records of patients to evaluate the incidence of second tumours in patients having received radiation therapy in the past.
2) Use of biochemical indicators and markers to evaluate delayed damage during patient follow-up.
3) Continued study of biochemical indicators to determine the damage caused by different types of dose administration.

III. Progress achieved:

In the Radiotherapy Sections of the University of Florence and public hospital (USL 10D) in collaboration with the Radiation Biology Laboratory research has been carried out during the course of several years to evaluate the damage caused by radiation on healthy tissues and tumours.
This study concerns the analysis of biochemical, morphological indicators and cell kinetics in order to quantify acute and delayed damage resulting from ionizing radiation treatment.
Patients who were treated with radiotherapy in the past for tumours in various parts of the body are called for check-ups at predetermined intervals to evaluate any delayed damage or complications including the eventual appearance of a second tumour.
Numerous case studies are available of patients with epidermoid carcinomas of the head and neck irradiated according to conventional fractionation (CF) 2Gy/die, 5 days a week, to a total dose of 60 Gy.
During the last 10 years some multiple fractionations (MF) have been used to improve the results of the treatment.
Our research on laboratory animals had shown good tolerance in highly proliferative tissues after MF of 2 Gy every 8 hours and 3 Gy every 12 hours to a total dose of 6 or 12 Gy.
Also a MF of 3x2 Gy every 4 hours, followed by a second series of 3 fractions after an interval of 16 hours, had shown good tolerance in the small intestine of rats.
In patients with head and neck tumours the following schedules have been used: a) 3x2 Gy/die every 4 hours, 5 days a week to a total dose of 48 Gy, b) 2x2 Gy every 8 hours 5 days a week to a total dose of 52 Gy, c) 3x1 Gy every 4 hours to a total dose of 60 Gy.
In the 4 groups of patients biochemical indicators showed different modifications. Tissue Polypeptide Antigen (TPA) showed a statistically significant increase with respect to healthy subjects. After irradiation, TPA produced by the duct cells of the salivary glands and by neoplastic tissue increased to 6-7 times the value before irradiation. A linear correlation between the daily dose and the increase of TPA ($r=0.971$) was observed after the first day. A similar correlation is evidenced for the amylase activity ($r=0.995$) as indicator of acinar cell injury. We are now investigating if the ratio of the TPA values before and at the end of therapy can be used as a marker of the evolution of the neoplasia.

The patients included in the follow-up study were affected by ENT tumours and treated with curative radiotherapy between 1960 and 1984. The distribution of the cases according to the site of primary tumour was as follows: 229 oral cavity, 223 nasopharynx, 282 oropharynx, 360 larynx, 80 hypopharynx, 60 salivary glands and 69 paranasal synuses.
Among these cases we have found 52 metachronous second tumours. The majority of the second primary tumours appeared in distant regions (41) and 11 in the ENT region. The time interval between the end of treatment and the diagnosis of the second ENT primary tumours ranged from 1 to 12 years. We are continuing our study to determine any possible correlation between radiation treatment and the appearance of a second tumour.
Moreover the follow-up of 457 patients treated with 131I for thyroid carcinoma from January 1, 1977 to December 31, 1987 has been revised . The preliminary results of these study showed: a) 25 patients had died of thyroid carcinoma or other non neoplastic diseases; b) 39 cases were lost during the follow-up; c) 5 patients showed a second primary tumour. Four of these are affected respectively by a carcinoma (localized in breast, kidney, badder or prostate) appeared during a period of 2-6 years after 131I administration. The fifth case, 60 years old at time of treatment, received 2 doses of 131I (75 and 150 mCi respectively) with a 19 month interval. An ALL appeared 9 years after the first 131I administration.

IV. Objectives for the next reporting period:

In the future we propose to carry out a more detailed statistical analysis of clinical record data in follow-up patients having undergone radiation therapy. Also case studies of patients irradiated on the abdomen as treatment for gynecological tumours will be examined. The research on biochemical and biological indicators and markers will continue in order to evaluate the acute and delayed modifications caused by ionizing radiations and to determine any eventual correlation between radiation therapy and the appearance of a second tumour.

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

- **Sezione di Medicina Nucleare - Dipart. di Fisiopatologia Clinica, Università di Firenze**
- **Cattedra di Radioterapia, Istituto di Radiologia, Università di Modena**

VI. Publications:

A. Becciolini, S. Porciani, A. Lanini, A. Chiavacci, E. Cellai
EFFECT OF CONVENTIONAL AND MULTIPLE FRACTIONATION TREATMENTS ON THE SERUM AMYLASE ACTIVITY.
Acta Oncol. 26,139-142, 1987

S. Porciani, A. Becciolini, R. Bandinelli, A. Chiavacci, E. Cellai, P. Ponticelli, R. Mungai
VALORI DI OLIGOELEMENTI IN PAZIENTI NEOPLASTICI E LORO MODIFICAZIONI IN CORSO DI RADIOTERAPIA.
Giorn. It. Chim. Clin. 12,113-118, 1987

A. Becciolini, M.S. Tommasi, S. Porciani, B. Fantappiè, E. Cellai, A. Chiavacci
SERUM TISSUE POLYPEPTIDE ANTIGEN (TPA): MARKER OF ACUTE INJURY ON SALIVARY GLANDS DURING RADIATION THERAPY.
Int.J.Radiat.Oncol.Biol.Phys. 13,1339-1342, 1987

Short Communications :

E. Scubla, M. Balzi, P. Boanini, M. Laddaga, M.G. Fabrini, A. Chiavacci, D. Cremonini, A. Becciolini
PARAMETERS OF CELL KINETICS IN HUMAN ORAL CAVITY CARCINOMAS AFTER IRRADIATION.
Int.J.Radiat.Biol. 51,922, 1987

M.S. Tommasi, S. Porciani, B. Fantappiè, R. Cellai, A. Becciolini
SERUM TISSUE POLYPEPTIDE ANTIGEN (TPA) AND PLASMA AMYLASE ACTIVITY AS EARLY BIOCHEMICAL INDICATORS OF RADIATION INJURY TO SALIVARY GLANDS.
Int.J.Radiat.Biol. 51,922-923, 1987

S. Porciani, A. Becciolini, A. Lanini, A. Chiavacci, E. Cellai, S. Magrini
POLYAMINES IN HEAD AND NECK CANCER: EFFECTS OF RADIATION THERAPY.
In: Radiation Research, E.M. Fielden, J.F. Fowler, J.H. Hendry and D. Scott Eds., Taylor and Francis, London, vol. 1, 292, 1987

M. Balzi, A. Becciolini, E. Scubla, A. Chiavacci, E. Zanieri, E. Cellai
THE BEHAVIOUR OF L.I. IN ORAL CAVITY CARCINOMAS FOLLOWING RADIOTHERAPY.
In: Radiation Research, E.M. Fielden, J.F. Fowler, J.H. Hendry and D. Scott Eds., Taylor and Francis, London, vol. 1,193, 1987

A. Becciolini, S. Porciani, E. Cellai, P. Olmi, M. Tommasi
GLI INDICATORI BIOCHIMICI DEL DANNO DA RADIAZIONE.
Atti XVII Congr.Naz. AIRB, Modena, pag. 25, 1987

M. Laddaga, M.G. Fabrini, F. Cartei, M. Balzi, G. Di Candio, R. Pingitore, A. Becciolini
MODIFICAZIONI MORFOSTRUTTURALI DA RADIOTERAPIA NEI TUMORI DEL RETTO.
Atti XVII Congr.Naz. QIRB, Modena, 1987, pag. 37

M. Balzi, E. Scubla, P. Boanini, A. Chiavacci, M. Laddaga, M.G. Fabrini, E. Zanieri, A. Becciolini
MODIFICAZIONI DELL'ATTIVITA' PROLIFERATIVA DURANTE LA RADIOTERAPIA IN TUMORI DELL'OROFARINGE.
Atti XVII Congr.Naz. AIRB, Modena, 1987, pag. 66

A. Porciani, A. Lanini, M. Attanasio, L. bruschini, A. Becciolini
EFFETTI DELLE RADIAZIONI SULLE POLIAMINE TISSUTALI.
Atti XVII Congr.Naz. AIRB, Modena, 1987, pag. 76

A. Becciolini, M.S. Tommasi, S. Porciani
MODIFICAZIONI DEL TPS IN RELAZIONE ALLA RADIOTERAPIA
Abstr. 3rd Int.Congr. on Tumor Markers, Napoli, 1987, pag. 102

G.P. Biti, E. Cellai, R. Santoni
INCIDENZA DEI SECONDI TUMORI IN SOGGETTI SOTTOPOSTI A RADIOTERAPIA.
Atti XVII Congr.Naz. AIRB, Modena, 1987, pag. 44

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-072-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. 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.: B16-D-072-NL

Role of oncogenes in malignant transformation of mouse cells.

Head(s) of project:

Dr. P.A.J. Bentvelzen
Radiobiological Institute TNO
Division of Health Research TNO
Lange Kleiweg 151
P.O. Box 5815
2280 HV Rijswijk

Scientific staff:

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 a putative oncogene rescued from the tumour cell line T-neo-1, which has been arisen after exposure of NIH/3T3 cells to irradiated mouse DNA and plasmids containing the long terminal repeat of the Moloney murine leukaemia virus as well as plasmids containing the selectable marker neo^R, confirming resistance to the neomycin analoque G418.

III. Progress achieved:

Two plasmids, rescued from the T-neo-1 line and containing the long terminal repeat (LTR) of Moloney murine leukaemia virus, had transforming activity on NIH/3T3 cells almost equal to the plasmid pT24, containing the activated H-ras oncogene isolated from the human bladder carcinoma line T24. The resulting NIH/3T3 transformants contained plasmid DNA sequences, as revealed by Southern blot analysis, indicating that transformation was due to the uptake of administered DNA. The inserted mouse DNA sequences in the two transforming plasmids had a size of only 3.5 kb.

The rescued sequence, called tno, proved by Southern blot hybridization to be amplified in the T-neo-1 line, although the additional bands (see Fig. 1) represent submolar quantities. This suggests that T-neo-1 would be a mix of transformants, in which the rescued sequence has been integrated at different sites.

The tno sequence is expressed in the T-neo-1 line by an RNA species of 2 kb, as revealed by Northern blot hybridization (Fig. 2). In this line, using the LTR of Moloney murine leukaemia virus as a probe, a high rate of expression of the LTR is found. The T-neo-1 line contains at least six additional LTR's. The variety of RNA molecules found in this Northern blot with the LTR probe indicate that the different integrated LTR's drive the expression of neighbouring sequences. Presumably one LTR is co-expressed with the tno sequence. This suggest that the tno-specific RNA sequence is considerably smaller than 2 kb and the 3.5 kb of the insert in the rescued plasmid.

The tno sequence gave under stringent hybridization conditions a signal in Southern blots with DNA not only from mice and rats but also from dogs, sheep, cattle, trouts and chickens, indicating this sequence to be highly conserved in DNA from vertebrates. In DNA of mice, rats and cattle multiple copies of this sequence were present.

No homology was found by dot blot hybridization with the following cloned oncogenes: abl, erb-B, fes, fms, fos, int-1, myb, myc, pim-1, H-ras, K-ras, N-ras, raf, rel, ros, sis, src or ves. This suggests tno to be a novel oncogene.

In 17 other NIH/3T3 or BALB/3T3 lines thought to be transformed by mouse DNA fragments no amplification of this tno sequence was found, suggesting that the oncogen is not involved in the transforming event giving rise to these tumour lines.

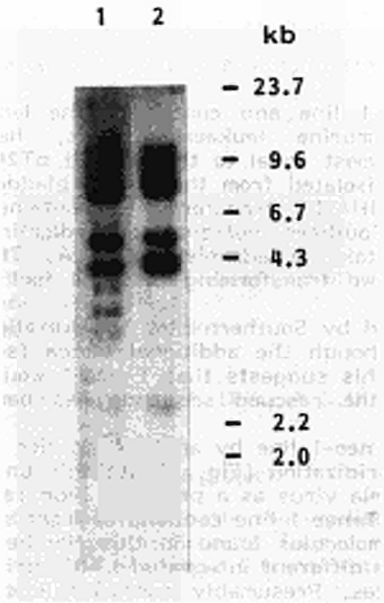

Figure 1:
Southern blot analysis of DNA from (1) T-neo-1 and (2) control NIH/3T3 cells with the rescued tno sequence as probe.

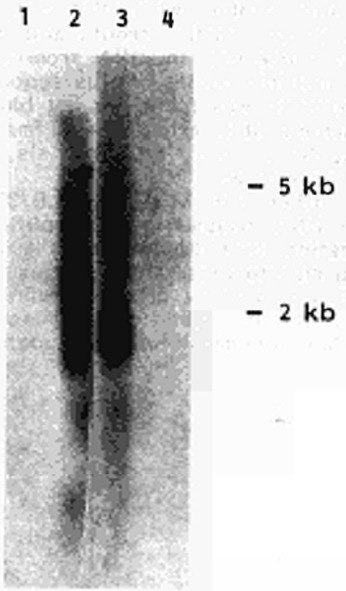

Figure 2:
Northern blot analysis of RNA from T-neo-1 (lane 2 and 3) and control NIH/3T3 cells (lanes 1 and 4) with the LTR of Moloney murine leukaemic virus (lanes 1 and 2) or the rescued tno sequence (lanes 3 and 4) as probe.

IV. Objectives for the next reporting period:

The rescued mouse DNA inserts will be sequenced in order to get insight into the organization of tno. The sequences will be compared with that of other known sequences, in particular of proto-oncogenes and other genes, associated with growth control. The expression of tno in a great battery of radiation-induced rodent tumours will be tested by Northern blot analysis. In case of expression it will be investigated by means of Southern blot analysis, whether this is accompanied by amplification or translocation of tno.

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

VI. Publications.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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% of the irradiated monkeys have died compared with 40% 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 third contract year two untreated control monkeys and two X-irradiated animals died. One monkey of the latter group died with malignant lesions. In the present communication the necropsy results of the four 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.

During 1987 two untreated control monkeys and two X-irradiated animals died.

The non-irradiated male monkey (case number 1747), born in 1966, died in March 1987 of acute right cardiac failure. At necropsy and histology, an extreme hyperplasia of the muscular wall of the large pulmonary arteries was observed compatible with a diagnosis of pulmonary hypertension. The right cardiac centricle was dilated and the liver showed signs of acute congestion. Tumours were not observed.

The non-irradiated male monkey (Balthazar), born in 1961, died in February 1987, due to respiratory failure. This monkey had an excessive thoracic kyphosis at Th 5-6 for yours. At necropsy, severe lung lesions were present. There was extensive lungmite infection with bronchiectasis, lung fibrosis and emphysema. The large systemic arteries showed focal intimal fibrosis and a variable narrowing of the lumen. Left and right cardicac ventricles were hypertrophic. Other lesions observed were multifocal, fibrotic thickening of the liver capsule with periportal and perisinusoidal fibrosis in the subcapsular area and chronic gastritis. Tumours were not observed.

The X-irradiated female monkey (case number 2490), born in 1971, was euthanized in April 1987, because of a technically inoperable uterus myomatosus. Necropsy and histological examination revealed, in addition to the presence of multiple benign leiomyomas of uterine body and cervix, also a cavernous hemangioma of the left adrinal, a benign splenic stromal tumour, a severe chronic gastritis and fibrosis and mineralization of the pancreatic islets.

The X-irradiated female moneky (case number 2489), born in 1971, was euthanized in October 1987 because of increasing weightloss and intractable diabetes. Necropsy and histological lesions compatible with long-standing diabetes were observed. The liver showed multifocal areas of fatty change. In the lungs, focal mild interstitial fibrosis was found. No neoplastic lesions were observed.

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 2 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 necropsis 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, Europan collaboration has been established within the framework of the European Late Effect Project Group (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, 1978.

Broerse, J.J. Hollander, C.F. and Van Zwieten, M.J.: Tumour induction in Rhesus monkeys after total body irradiation with X-rays and fission neutrons. Int. J. Radiat. Biol. 40, 671, 1981.

Broerse, J.J., Van Zwieten, M.J. and Zurcher, C.: Carcinogenic risk of the medical use of X rays. Proc. 6th Annual Meeting of the European Society for Therapeutic Radiology and Oncology (ESTRO), 222, Lisboa, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-219-NL

Academisch Ziekenhuis Leiden
Rijnsburgerweg, 10
NL - 2333 AA Leiden

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

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Afd. Klinische Oncologie K 1-48
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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, Drs. L.A. Hennen and Ing. J. Weeda.

I. Objectives of the project:

Quantitative estimates of the risk for breast cancer indcution are essential for risk-benefit-cost analysis of mammography procedures. Large scale programmes on radiation-induced mammary neoplasms haven 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 estabished between 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) will be analyzed. Different methods and models will be 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 analysis is performed with product-limit methods, sum-limit methods and Weibull distribution functions.

III. Progress achieved:

Cancer induction is generally considered to be the most important somatic effect of ionizing radiation. It is therefore of great concert to assess the quantitative risk on exposure to radiations of different quality and to obtain information of the dose-response relationships for carcinogenesis. The female breast is one of the tissues with a relatively high sensitivity to the oncogenic influence of radiation.
Over the past decade, a number of experimental studies on mammary carcinogenesis in different rat strains have been performed at the Radiobiological Institute TNO. A first step to report tumour incidence data is to score the fraction of animals that develop a tumour or the mean number of tumours per animals observed after a given dose. However, this use of so-called uncensored data may lead to erroneous interpretation of results. During the observation time, the group of animals at risk may be reduced due to intercurrent deaths or other losses of animals from the experiment due to reasons not related to the endpoint under investigation. Consequently, one deals with incomplete right censored data. Appropriate corrections for competing risks in the tumour rate analysis can be made by using life-table methods such as the product limit estimate of Kaplan and Meier or the sum limit estimate. The resulting quantities, such as the cumulative tumour rate or the cumulative prevalence and actuarial incidence, provide meaningful expressions of the response.
The probability curves produced by the life table analysis inevitably show discontinuities in the course of time. The stochastic effect of carcinogenesis, however, can be described by hazard functions which result in curves or several groups with a common shape. This can be done using non-parametric models, e.g. the proportional hazards model (Kellerer and Chmelevsky, 1982) or analytical models, e.g. the Weibull distribution (Broerse et al., 1985). In the analysis performed at TNO, the time elapsing from when an animal enters the experiment until a specified type of tumour becomes palpable, is taken as the endpoint. One knows either the actual time that a tumour is observed in an animal or that the animal has not developed such a tumour up to its time of disappearance from the experiment. Methods of analysis that accommodate censoring are generally called failure time distributions. The onset time of a tumour, hereafter referred to as a failure, has been taken as the random variable. The proportion of animals surviving without evidence of tumour up to a certain time has been analyzed with a continuous parametric failure-time model. Specific corrections have been applied for the occurrence of microtumours which are only observed upon obduction (Broerse et al., 1986). The three parameter Weibull distribution can be used to described cancer failure times. Emperically, the model provides an adequate description of the power-of-age dependence of observed incidence rates for many adult human and animal carcinomas. The probability of surviving without evidence of tumour can be deseribed by the survivor function:

$$S(t) = \exp - \{(t-\gamma)/\alpha\}^{\beta}$$

where α is the time scale parameter, β the shape parameter and γ the location parameter (Broerse et al., 1985) The induction of carcinomas in WAG/Rij rats after X-irradiation and hormone administration has been analyzed according to this method (see Fig. 1).

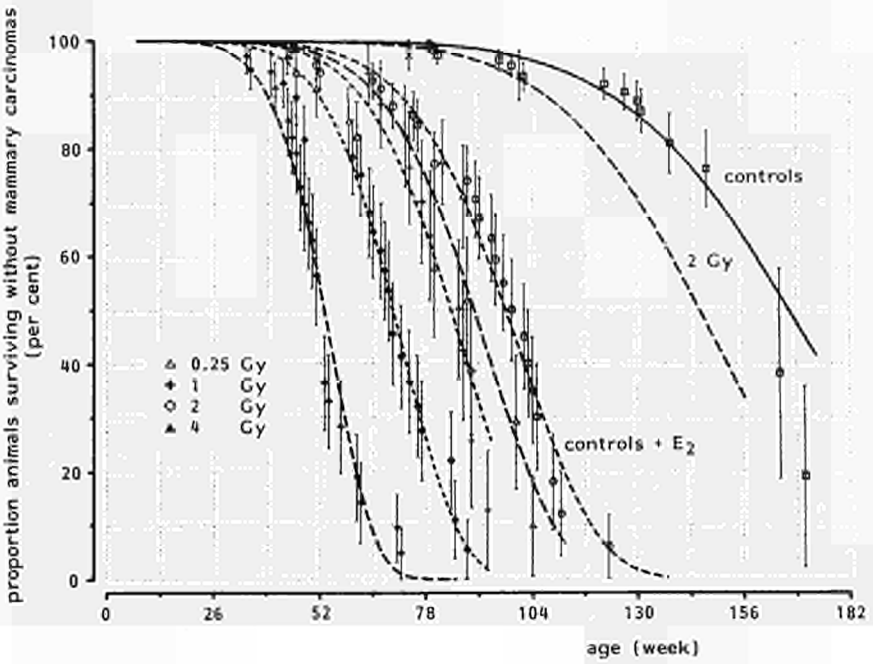

Figure 1. Probability of surviving without evidence of carcinomas in WAG/Rij rats after X-irradiation and administration of the hormone E_2 one week prior to irradiation. The two right hand curves refer to the response of the animals without hormone.

The relative excess hazard can be defined independently of the time function and describes the net effect in the hazard of an irradiated cohort relative to the hazard of the control cohort. The relative excess hazard is then equal to:

$$\eta(D) = \{\alpha(0)/\alpha(D)\}^{\beta} - 1$$

In a recent experiment the induction of mammary tumour in the rat have been investigated after fractionated low-dose gamma irradiation. According to the above described formalism the relative excess hazard has been calculated as a function of the total absorbed dose (see Fig. 2).
Non-parametric models such as employed by Kellerer and Chmelevsky (1982) postulate no analytical expressions for the time or dose dependences of tumour rates. This proportional hazards model admits any dependence of the cumulative tumour rates on time with the only constraint that it may not decrease with time. The non-parametric model is attractive because it introduces a minimum of a priori assumptions concerning the time or dose dependence of tumour rates. The Würzburg computer programmes, written in PASCAL, as provided by Chmelevsky et al. have been impemented and adapted to a MS-DOS microcomputer. The available tumour incidence date are subjected to the different methods.

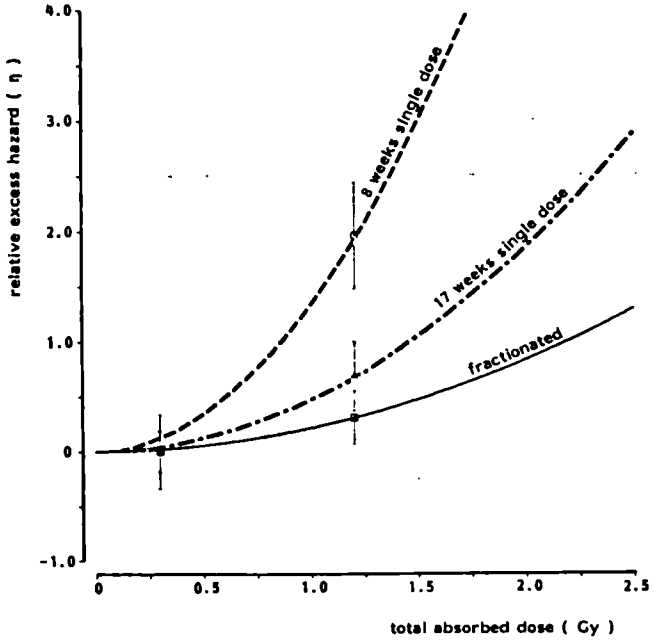

Figure 2. Relative excess hazard as a function of the total absorbed dose for the induction of mammary carcinomas in WAG/Rij rats after gamma-irradiation.

IV. Objectives for the next reporting period:

Rats from different strains have been exposed to single and fractionated irradiation with X-rays, gamma rays and mono-energetic neutrons with energies of 15, 4 and 0.4 MeV. For specific groups the radiation treatments were combined with the administration of hormones. These experimental data will be analyzed by product-limit estimates, actuarial methods and Weibull distribution functions.

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

- Institute für Medische 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 (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 aniamls 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.

Broerse, J.J. Hennen, L.A., Klapwijk, W.M. and Solleveld, H.A.: Mammary carcinogenesis in different rat strains after irradiation and hormone administration. Int. J. Radiat. Biol. 51, 1091, 1987.

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.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
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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. BERARD, M.H. HENGE-NAPOLI, E. RONGIER

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".

Intoxication par voie intratrachéale de rats avec des composés industriels d'uranium.

Suivi des paramètres biologiques urinaires sur les animaux après intoxication par voie intraveineuse.

Sur l'homme, essai de corrélation entre les études de poste et les résultats de radiotoxicologie.

III. Progress achieved:

III.1 - Etudes "in vitro"

III.1.1 - Méthodology

Les composés étudiés principalement sont UF_4 et les Uranates calcinés sous forme UO_3 et U_3O_8. Des composés étalons ont été utilisés (UO_2F_2, UO_3 et U_3O_8).

2 tests in vitro ont été comparés :

- cellule à flux parallèle (modèle de la Lovelace) ou Dynamique.

- cellule d'essais statiques (contact directe de la poussière avec le liquide de solubilisation).

Les liquides de solubilisation ayant servi sont :

- Gamble,
- Carbonates à diverses concentrations,
- Phosphates,
- eau.

Les cinétiques sont exprimées sous forme de % non dissout en fonction du temps et ceci sous forme d'une somme d'exponentielles du type =

$$Y = \% \text{ Un.diss} = \sum Ai \; e^{-0.693\ t/Ti}$$

Ai = % du composé D, W ou Y
Ti = temps de demi vie.

III.1.2 - Results

Nous avons obtenu 2 types de résultats :

1) UF_4
L'étude a été menée sur les 2 tests avec 6 solutions de solubilisation. 3 concentrations en carbonates, 1 phosphate, Eau et Gamble. En règle générale les carbonates ainsi que l'eau favorisent la solubilisation d'UF_4 (comportement D à W). Par contre dans les phosphates et Gamble, l'UF_4 à un comportement de type Y.
Notons qu'un composé appartient toujours à plusieurs classes - exemple = UF_4 dans Gamble ou essai Dynamique :

$$Y = 0.17 \exp^{-0.693\ t/0.6} + 0.83 \exp^{-0.693\ t/230}$$

Les essais statiques suivent bien les essais dynamiques et donnent toujours des résultats un peu plus forts.

2) Uranates calcinés
Une étude physico chimique a été faite sur des composés industriels mal définis (dénomination RTZ, QML, NUFCOR).
Les résultats ont montré que RTZ et QML s'apparentaient plutôt à U_3O_8 + UaOb alors que NUFCOR serait un mélange du type UO_3 + UaOb.

Les 2 tests ont été utilisés avec Gamble, eau, Carbonates et Phosphates. Dans Gamble RTZ et QML sont de type Y et NUFCOR de tendance W à Y. Les carbonates semblent favoriser la dissolution de ces 3 composés (tendance classe W) et par contre les phosphates donnent une tendance Y.

III.2 - Etudes in vivo

III.2.1 - Methodology

L'effet néphrotoxique de l'uranium a été suivi en mesurant l'excrétion quotidienne de gamma glutamyl transférase chez des rats intoxiqués par voie intraveineuse à des doses de nitrate d'uranyle variant de 0,005 à 0,75 mg/kg.

Parallèlement, l'excrétion d'uranium urinaire est dosée par fluorimétrie.

D'autre part, la cinétique d'excrétion de composés d'uranium industriels a été suivie chez des rats après dépôt intra-trachéal.

III.2.2 - Results

Excrétion de la Gamma GT urinaire :
On observe une très grande différence entre l'excrétion des Gamma GT des mâles et des femelles :

- valeurs témoins mâles : 6,6 $\pm$ 2,9 unités/24 h (n = 24)
- valeurs témoins femelles : 2,06 $\pm$ 0,6 unités/24 h (n = 24)

Après intoxication, l'excrétion urinaire de Gamma GT est beaucoup plus importante chez les femelles que chez les mâles.

Pour les deux sexes, l'augmentation de l'excrétion de la Gamma GT devient significative pour des doses injectées supérieures à 30 µg/kg et s'accroît en fonction de la dose injectée.

Excrétion d'uranium après intoxication Intratrachéale par un uranate calciné : Le NUFCOR

Ce composé a été excrété suivant une cinétique de composé D à tendance W de façon comparable à certains résultats obtenus in vitro en présence de carbonates.

III.3 - Etudes sur l'homme

III.3.1 - Methodology

- résultats radiotoxicologiques de personnels exposés (rétention pulmonaire et excrétions urinaires et fécales).
- Résultats des contrôles atmosphériques journaliers (APA).
- Résultats des mesures des aérosols aux postes de travail (en particulier, diamètres aérodynamiques moyens).

Sur la base du modèle défini par la C.I.P.R. 30 et des données calculées par le Docteur PIECHOWSKI (DPS/SEAPS), le SHI a conçu un programme, implanté sur un microordinateur, permettant le calcul des incorporations soit lors d'une exposition chronique soit après une exposition unique, en tenant compte des caractéristiques du poste de travail et des résultats radiotoxicologiques.

III.3.2 - Results

Nous avons étudié plus particulièrement deux postes de travail et les agents affectés =

. exposition à des uranates au poste d'échantillonnage ; les aérosols sont des composés de classe W, d'un diamètre aérodynamique moyen de 8 micromètres. A ce poste les agents ont une surveillance hebdomadaire en uranium urinaire.

. exposition à des oxydes d'uranium, pour lequel la surveillance radiotoxicologique est centrée sur l'anthropogammamétrie et les analyses fécales.

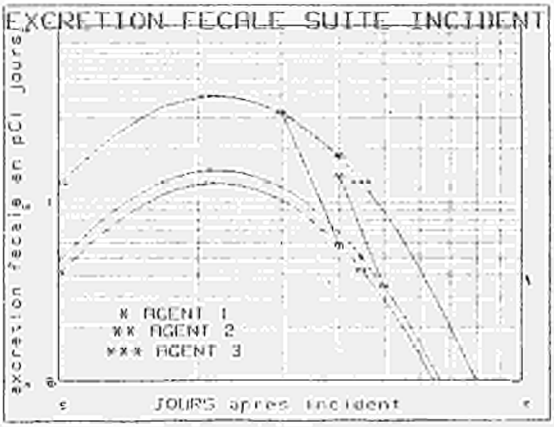
Nous présentons, ci-dessous, deux exemples de calcul :

a - Calcul de la dose interne après incident

Les hypothèses du calcul sont :

. exposition unique après incident
. U_3O_8, composé de classe Y
. diamètre aérodynamique moyen = 1 µm
. composition isotopique = 234 U, 235 U, 238 U.

Les calculs sont effectués à partir des résultats observés sur les selles.



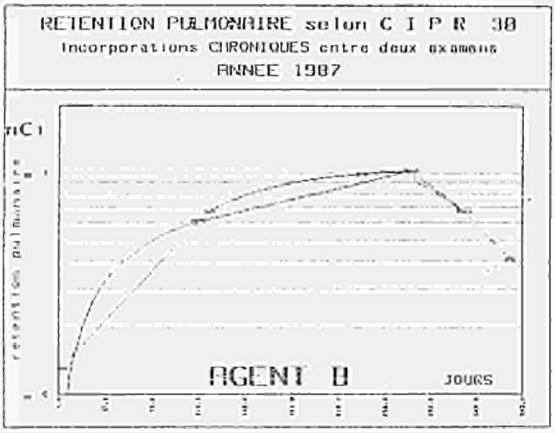
Les doses effectives sont comprises entre 0,10 et 0,32 millisievert pour les trois agents.

b - Calcul de la dose interne lors d'une exposition chronique pour un agent

Les hypothèses du calcul sont :

. exposition chronique pendant l'intervalle de surveillance,
. composé de classe W
. diamètre aérodynamique moyen = 1 µm
. composition isotopique = 234 U - 238 U - 235 U.

Les calculs sont effectués à partir des résultats de la rétention pulmonaire mesurée par anthropogammamétrie.



L'équivalent de dose effective est de 1,5 rem pour l'agent B.

III.3 Discussion

- Essais "in vitro"

Ces essais soulèvent le problème du choix d'une solution de solubilisation et montrent la complexité des phénomènes chimiques mis en jeu.

Il est très important de souligner l'expression des résultats sous forme de somme d'exponentielles, c'est-à-dire qu'un composé se divise en pourcentage de plusieurs composés de classe D, W ou Y. Les essais sont poursuivis avec l'étude de l'influence d'apport gazeux type CO2 et O2.

- Essais "in vivo"

La mesure de l'activité de la gamma glutamyl transférase urinaire s'est avérée un paramètre fiable et sensible de mesure de l'intoxication à l'uranium. Cette étude sera complétée par la mesure d'une autre enzyme urinaire, la N-acétyl-glucosaminidase.

La sensibilité de ces mesures sera testée après intoxication de rats par inhalation à des uranates industriels.

- Etudes sur l'homme

Les résultats radiotoxicologiques permettent de calculer les équivalents de dose effective en chronique et également en cas d'incident.

IV. Objectives for the next reporting period:

Les essais "in vivo" et "in vitro" seront poursuivis afin d'étalonner les systèmes "in vitro".

Les essais de corrélation entre les études de poste et les résultats de radiotoxicologie seront poursuivis.

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

- Docteur BEAU - DPS/SEGP - CEA - FONTENAY AUX ROSES - FRANCE

- Professeur GALLE - Laboratoire de biophysique - Université de PARIS - VAL DE MARNE - Faculté de Médecine - 94010 CRETEIL FRANCE

VI. Publications:

- HENGE-NAPOLI M.H., RONGIER E., CHALABREYSSE J.
  Suivi de la Gamma GT urinaire après intoxication au nitrate d'uranyle chez le rat. - Communication aux 3ème Journées Scientifiques de Suze-la-Rousse, les 16, 17, 18 septembre 1987.

- VERDERA J.M.
  Contribution à l'étude de la solubilisation des oxydes d'uranium - Ecole Centrale Paris - Thèse soutenue le 29 juin 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 study the long-term kinetics of alveolar clearance of colloidal gold particles, following microinjection into subpleural alveoli of rat lung. Lung tissue would be obtained from serial sacrifices for radioassay, as well as for autoradiography and electron microscopy, to provide information on the mechanisms of clearance of particles from, and redistribution within, the respiratory tract.

III. Progress achieved:

Methodology

A long-term study of alveolar clearance was commenced. 38 male F-344 rats were successfully injected with particles of colloidal gold, using the procedure previously described (G. Patrick and C. Stirling, J.Appl.Physiol. 60, 307-310, 1986). Glass micro-pipettes were used, with a bevelled tip of outside diameter 10-14μm, for the micropuncture of the left lung through the parietal pleura.

The colloid was prepared by reduction of $H^{195}AuCl_4$ with ascorbic acid so as to contain 3.3 μg Au/μl with a specific activity of 23-37 kBq/μg. The amount injected into each rat was not greater than 1 μg Au in 0.3 μl suspension. It was confirmed by gel chromatography that the colloidal preparation contained no soluble (ionic) gold.

For each rat the initial lung burden was assayed by thorax counting in a small animal whole-body counter. 5 rats were injected for subsequent sacrifice at 6 time intervals from 4 min to 9 months. An additional 8 rats are being repeatedly monitored for retained ^{195}Au by thorax counting over 18 months. Excretion data are being obtained using metabolism cages.

At sacrifice the rats were thorax-counted before and after removing the lungs, to estimate the proportion of the thorax count due to the remaining lung burden. Subsequently the left lung was divided along its major axis into 2-mm slices. These were assayed for ^{195}Au and selected slices were either sectioned for autoradiography or were cut into 2-mm cubes for further processing for electron microscopy.

Results

Serial sacrifices have been completed for periods up to 4 months, and partially completed for the 9-month group. The ratio of lung activity to whole thorax activity at sacrifice has not so far varied significantly with time; the overall mean was 0.964 ± 0.009.

Repeated thorax counts on the 18-month group have now been completed to 39 weeks. The lung burdens at selected times are provisionally estimated as in Table 1.

Of the total ^{195}Au excreted during the 4th and 8th weeks after injection, the urine contained 5.3 + 1.5 and 1.11 + 0.57 % respectively. The proportion of gold particles cleared to the mediastinal lymph nodes after 4 months was 0.57 + 0.16 % of the initial deposit.

Table 1 ^{195}Au lung burden as per cent of initial deposit, corrected for radioactive decay

Time (weeks)	Mean	SEM	Range
1	91	2.5	83-100
2	86	1.5	83-93
5	79	1.5	72-85
10	70	2.2	60-78
20	66	3.7	53-80
39	56	4.2	45-73

Preliminary analysis of the distribution of ^{195}Au between slices of the left lung confirmed that the initial deposit was confined to within 1-2 mm along its length. This pattern was found to be not markedly different at later times, up to 4 months after injection. The distribution across the slices, i.e. between the 2-mm cubes, is currently under study; autoradiographic studies and electron microscopy of the lungs are also in progress.

Discussion

The long-term study is not yet complete, but some tentative conclusions can be drawn.

There was no fraction of gold particles rapidly cleared from the subpleural site. The lung retention curve does not appear to fit a single exponential term, but a definitive expression must await observations at later time points.

Only a small proportion of the gold is cleared via the urine at early times after deposition, as would be expected. It will however be of great interest to observe the excretion pattern at later times, when the clearance rate is at its lowest.

Even at long times after injection there is no wide-scale redistribution of particles throughout the lung. The assessment of any long-term retention of particles in association with the airway walls, whose epithelium contains cells at risk of carcinogenic transformation, must await detailed analysis by autoradiography and electron microscopy.

IV. Objectives for the next reporting period:

The long-term study of gold particle clearance from rat lung alveoli will be continued, as indicated above. Excretion of gold will be analysed into particulate clearance and dissolution of gold particles. Further micro-injections will be made to expand this investigation into alveolar clearance mechanisms.

An investigation will be made to see if the long-term retention of particles in association with the large airways of the rat can be satisfactorily measured 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:

Takahashi, S. and Patrick, G., Long-term retention of ^{133}Ba in the rat trachea following local administration as barium sulfate particles. Radiation Research 110, 321-328, 1987.

Takahashi, S. and Patrick, G., Patterns of lymphatic drainage to individual thoracic and cervical lymph nodes in the rat. Laboratory Animals 21, 31-34, 1987.

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 as they became available for the distribution of tourmaline-containing particles. Tourmaline is a component of the rock dust which these workers will have inhaled occupationally. The comparison of tourmaline clearance from rat lung with $^{239}PuO_2$ clearance rates was to be continued, to validate tourmaline as a model for PuO_2 in man. The distribution of UO_2 particles in rat lung was to extended to animals killed two years after inhalation. The cytotoxic effect of uranium on pulmonary macrophages was to be investigated up to 90 days after inhalation.

III. Progress achieved:

Methodology

For the study of the distribution of tourmaline in sections of lungs from tin miners, the autoradiographic technique using CR-39 has been developed to provide a new method of imaging the lung tissue itself on the plastic; this improves on the alpha-shadowing technique of D.J. Gore et al. (Phys. Med. Biol. 23, 149, 1978) regarding resolution, convenience and reliability. The lung section was mounted on plastic and soaked for an hour in 2% $Na_2B_4O_7$, then dehydrated and dried. A pre-etched top slide of CR-39 was clamped over the section; they were then exposed to a thermal neutron fluence of $5 \times 10^{12} cm^{-2}$. After etching, the top plate revealed a detailed image of lung tissue. The tissue section itself is preserved by this method, and can be stained to provide more detailed histological information.

The exposure of Fischer-344 rats by nose only to an aerosol of neutron-activated rock dust was described in the previous report. The animals were killed in groups of four at 3, 7, 30, 90 and 180 days after inhalation, and the lungs, livers and spleens dissected free.

The study has continued of rats exposed to an aerosol of ^{235}U-enriched UO_2, as described in the previous report. All the animals have now been killed at intervals up to two years after inhalation. Organs have been analysed for UO_2 by gamma counting and by delayed neutron analysis. The lungs have been sectioned for autoradiography on CR-39.

For the study of the effect of UO_2 on pulmonary macrophages by electron microscopy, further groups of rats have been exposed to the UO_2 aerosol and killed at 0, 7, 15, 30, 45, 60 and 90 days after inhalation. Lung clearance has also been measured in these animals.

Results and Discussion

No new post-mortem lung specimens became available from tin miners. the improved autoradiographic technique using the CR-39 top plate has proved useful for studying the distribution in rodent lung tissue of actinide particles such as $^{235}UO_2$. Fission fragment 'stars' were readily distinguished in the neutron-induced top plate image.

In the study of tourmaline clearance from rat lung, ^{54}Mn was produced in the rock dust aerosol by neutron activation from iron present in the tourmaline. The lungs were analysed at sacrifice for ^{54}Mn by gamma counting with a Ge(Li) detector. Retained lung burdens have been

estimated up to 180 days after inhalation.

In the long-term study of clearance and distribution of UO_2 in rat lung, all the animals have now been killed. All the required autoradiographs on CR-39 have been produced, and the analysis of particle distribution is in progress using the Quantimet 720 image analyser. 58% of the initial UO_2 lung burden was retained in the body after 6 months, falling to 21% after 2 years; of the latter, 81% remained in the lungs and 10.5% was found in the thoracic lymph nodes. This material was cleared from rat lung at a slower rate than was found for the tourmaline dust.

In the rats exposed to UO_2 aerosol for the study of effects on macrophages, 82% of the initial lung burden was retained in the body after 90 days. Examination of lung sections by electron microscopy is in progress; evidence of cytotoxicity of the uranium particles has already been obtained.

IV. Objectives for the next reporting period:

Human lung specimens will be analysed for tourmaline distribution as they become available. The clearance rate of tourmaline aerosol from rat lung will be finally evaluated, and compared with corresponding clearance rates for inhaled insoluble actinide aerosols.

The study of the spatial distribution of UO_2 particles in autoradiographs of rat lung will be continued. The ultrastructure of pulmonary macrophages will continue to be studied in rats exposed to the same aerosol.

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

Harwell Laboratory (AERE);
Department of Physics, University of Bristol: Dr. D. L. Henshaw.
Atomic Weapons Fstablishment, Aldermaston.

VI. Publications:

Morris, K.J. and Batchelor, A.L., The location of boron-containing dust in the lung utilising neutron-induced autoradiography techniques with a CR-39 solid state track detector.
Physics in Medicine and Biology, 32, 1501-1508, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

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.

Scientific staff: Dumont, J.E., M.D., Ph.D., V. DE MAERTELAER, Ph.D. VAN SANDE, J., Ph.D., ROGER, P.P., Ph.D., HEPBURN, A., M.S., COCLET, J., M.S., REUSE, S., M.S., LAMY, F., Ph.D.

I. Objectives of the project:

1. Epidemiology : a) Retrospective study of patients exposed to X ray irradiation
   b) Retrospective study of patients irradiated with ^{131}I.
   c) Analysis of the consequences of Tchernobyl accident
2. Human thyroid tumors : a) Definition of the role of oncogenes in human thyroid carcinogenesis
   b) Definition of the regulation pattern of thyroid autonomous adenomas
3. Definition of cell kinetics of the human thyroid.
4. Development of a human thyroid cell culture model.

II. Objectives for the reporting period:

1. Epidemiology : a) Introduction of data on X ray irradiated patients in the computer data files.
   b) Pilot retrospective study of patients irradiated with ^{131}I at the Bordet Institute
2. Human thyroid tumors : a) search for the oncogene detected in one tumor from Villejuif.
   b) study of the phosphatidylinositol response in normal and adenomatous human thyroid tissue.
   c) Study of protooncogene expression in the dog model
3. Cell kinetics in human thyroids : study of anedomas
4. Development of a human thyroid cell in primary culture model.

III. Progress achieved

I. METHODOLOGY

1) Epidemiology

a) Introduction of data in computer data files

b) 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

2) Human thyroid tumors

a) Search for oncogene in Villejuif tumor. Repeated cycles of transfection of 3T3 cells. Check of human DNA presence by Alu DNA probes (Krontiris, T.G. & Cooper, G.M., Proc. Natl. Acad. Sci. USA 78 (2) 1181-1184, 1981; Jelinek, W.R. et al., Proc. Natl. Acad. Sci. USA, 77 (3) 1398-1402, 1980).

b) Study of the PI response : incubation of slices of normal and adenomatous tissue with 3H 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 (Graff, I., Mockel, J., Laurent, E., Erneux, C., Dumont, J.E., FEBS Letter, 210 (2), 204-210, 1987.

c) Labeled cDNA probes of vmyc and vfos are used to demonstrate by autoradiography of Northern blots the corresponding cmyc and cfos mRNA in extracts from dog thyroid cells (Reuse, S., Roger, P.P., Vassart, G., Dumont, J.E., Biochem. Biophys. Res. Commun. 141 (3), 1066-1076, 1986.

3) Cell kinetics in human thyroid. ^{3}H thymidine labeling for one hour of the slices from human adenomas obtained at surgery (Lab. Invest. 23, 635, 1970).

4) Development of human thyroid cell primary culture model : 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 3H thymidine labeled nuclei, or DNA measurement (Roger, P.P., Reuse, S., Servais, P., Van Heuverswyn, B., Dumont, J.E. Cancer Res., 46, 898-906, 1986).

II. RESULTS

1) Epidemiology

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 131I 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.

2) Human thyroid tumors

a) Villejuif patients oncogene : transfection of 3T3 by patients DNA was realized 4 times and gave positive foci : in each case (although with low frequency). Secondary transfection with DNA of these foci gave equivocal results. Human Alu DNA could not be demonstrated

b) 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.

c) Protooncogene expression : cmyc and cfos mRNA expression is enhanced in dog thyroid cells in primary culture in response to TSH, epidermal growth factor and phorbol esters, i.e. to the three major mitogenic agents acting on these cells. The kinetics of this expression is very different in the three cases. The method is now suitable for its application to human cells.

3) Cell kinetics. 7 adenomas have been studied. The labelling index is always higher than in normal tissue (59/83500). Calculation of the doubling time of adenomatous tissue suggests that several years are necessary to produce a 10^9cells (1 g) nodule.

4) Human thyroid cells in primary culture : the model developed for dog thyroid cells has been applied to human cells. 18 primary cultures have been carried out. When seeded in the presence of 1% serum the cells spread. In the absence of serum they remain aggregated in pseudofollicles. In the latter case, but not ine the former, the cells trap iodide. In both case the cells proliferate in response to TSH, EGF and phorbol esters.

III DISCUSSION

1) Epidemiology

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.

2) Human thyroid tumors

a) Search for oncogene in an anaplastic tumor. Primary foci of transformed cells have been obtained with the DNA of this tumor. However we have been unable to obtain significant second generation foci. This 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.

b) Phosphatidylinositol response. The results suggest that in thyroid hyperplasia coupled to hyperfunction, 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 hyperfunction adenomas of other tissues.

c) Protooncogene expression : the results obtained with the dog thyroid cell model allow now the extension of this work to human cells in primary culture.

3) Cell kinetics. The results obtained can be used in the analysis and interpretation of clinical data on human thyroid nodules development.

4) Human thyroid cells in primary culture : 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.

IV. Objectives for the next reporting period:

1) Epidemiology : a) Data gathering (controls) on Pisa and Villejuif patients
b) Enquiry on the feasibility of a joint multicenter study on normal patients having received I^{131} for diagnosis

2) Human thyroid tumors : a) extension of the preliminary results on the role of the imbalance between the cyclic AMP and the phosphatidyl inositol systems in the pathogenesis of hyperfunctioning adenomas
b) extension of the preliminary study of cell kinetics on thyroid tumors

3) Human cell culture : a) study of the role of paracrine factors (interleukin, somatomedins) or proliferation and differentiation expression
b) study of protooncogene expression in human thyroid cells in culture
c) search for ways to increase the lifespan of the cells to 10 generations
d) trial of various in vitro transformation methods (X rays, carcinogens)
e) trial of thyroid foetal cell culture

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

- Institut du Cancer, Villejuif, France
- Thyroid Research Unit, Universita di Pisa , Italia
- Service de Chirurgie, Institut Bordet, Bruxelles, Belgique
- College of Technology, Dublin, Ireland.

VI. Publications:

- REUSE, S., ROGER, P.P., VASSART, G., DUMONT, J.E.
Enhancement of cmyc mRNA concentration in dog thyrocytes initiating DNA synthesis in response to thyrotropin, forskolin, epidermal growth factor and phorbol myristate ester. Biochem. Biophys. Res. Commun. 141 (3), 1066-1076, 1986.

- ROGER, P., SERVAIS, SP., DUMONT, J.E.
Induction of DNA synthesis in dog thyrocytes in primary culture: synergistic effects of thyrotropin and cyclic AMP with epidermal growth factor and insulin. J. Cell. Physiol. 130, 58-67, 1987.

- REUSE, S., ROGER, P., LAMY,, F., FOUREAU, F., GERARD, C., DUMONT, J.E.
Control of thyroid cell proliferation : the example of the dog thyrocyte. Acta Endocrinol. (Copen.) Suppl. 281, 215-219,1987.

- DUMONT, J.E., ROGER, P., LUDGATE, M.
Autoimmunity and thyroid growth : Methods, concepts and misconceptions. Acta Endocrinol. 281, 299-301, 1987.

- LAURENT, E., MOCKEL, J., VAN SANDE, J., GRAFF, I., DUMONT, J.E.
Dual activation by thyrotropin of the phospholipase C and cyclic AMP cascades in human thyroid. Mol. Cell. Endocrinol. 52, 273-278, 1987.

- ROGER, P.P., SERVAIS, P., DUMONT, J.E.
Regulation of dog thyroid epithelial cell cycle by forskolin, an adenylate cyclase activator. Exp. Cell. Res. 172, 282-292, 1987

· VAN SANDE, J., LAMY, F., ROCMANS, P., COCHAUX, P., DUMONT, J.E.
The cyclic AMP system in thyroid autonomous nodules
J. Clinical Endocrinology 1988, in press.

· ROGER, P.P., DUMONT, J.E.
Thyrotropin is a potent growth factor for normal human thyroid cells in primary cultures. Biochem. Biophys. Res. Commun. 149, 707-711, 1987 (in press)

· DUMONT, J.E., LUDGATE, M., ROGER, P.
Assays for thyroid growth immunoglobulins : Methods concepts and misconceptions. Endocrine Rev. 8, 448-454, 1987. (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Fondation Bergonié
Cours de l'Argonne, 229
F - 33076 Bordeaux Cédex

Contract no.: B16-D-078-F

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

Dr. J.F. Duplan
Unité INSERM N° 117
Fondation Bergonié
Cours de l'Argonne, 229
F - 33076 Bordeaux Cédex

Telephone number: 056-91.16.61

Title of the research contract:

Mechanism of radiation-induced leukemogenesis and osteosarcomagenesis.

List of projects:

1. Mechanism of radiation-induced leukemogenesis and osteosarcomagenesis.

Title of the project no.:

B16-D-078-F
MECHANISM OF RADIATION-INDUCED LEUKEMOGENESIS

Head(s) of project:

E. LEGRAND, B. GUILLEMAIN

Scientific staff:

J.F. DUPLAN, T. ASTIER-GIN
Techniciens : 2

I. Objectives of the project:

The genome of some recombinant retroviruses which emerge during the process of radiation-induced leukemogenesis of the C57BL/6 mouse may be suspected to act as the causative factor or as a cofactor in this process. To test for this hypothesis we looked for new retroviral integration sites in the DNA of primary and transplanted tumors. Because retroviruses may induce leukemia by activating cellular genes adjacent to the integration site, we looked in tumor DNAs for the presence of a limited number of integration sites.

II. Objectives for the reporting period:

To examine, in primary and transplanted radio-induced thymic lymphosarcomas, the eventual presence of new retroviral insertions.

To determine the number, if any, of new retroviral integration sites and their identity in different tumors.

To molecularly clone the new recombinant retroviruses as well as flanking sequences.

III. Progress achieved:

In our last progress report we summarized experiments aimed at the detection of new retroviral sequences in DNA of primary and transplanted radio-induced (4 x 1.75 Gy) thymic lymphomas. DNAs from such tumors were hydrolysed by Kpnl and analysed by the Southern method with a probe specific of the env gene of ecotropic viruses. In control C57BL/6 DNA, this probe detects a 4.0 kbp internal fragment of the endogenous ecotropic virus. Recombinant B-ecotropic retroviruses have an additional Kpnl site in their genome thus generating a shorter fragment (3.5 kbp) recognized with the probe. At that time we found such a fragment in radio-induced tumors, but only after transplantation.

In contrast to these previous experiments we report now the detection of new recombinant ecotropic sequences **in primary tumors** (28 %). This new finding may best be explained by the use of a probe having a high homology with the C57BL/6 ecotropic endogenous sequences as well as more sensitive techniques. The existence of a 3.5 kbp Kpnl restriction fragment in the digested DNA is indicative of the integration of an ecotropic provirus recombinant in the gag-pol region and thus very similar to the B-ecotropic recombinant retroviruses isolated by Rassart et al. (1983) in radio-induced thymoma derived cell lines. Our results obtained with primary tumors parallel those obtained with transplanted tumors.

By use of different restriction enzymes (EcoRl, HindIII) we could also show that, in most primary and transplanted radio-induced tumors harboring new recombinant proviral sequences, such sequences were inserted at one or a few sites per tumor. This demonstrates the clonal or oligoclonal origin of the tumors.

The same findings were obtained in our model associating the injection of T1223/B virus (a B-ecotropic recombinant retrovirus) and a subleukemogenic dose of radiation (2 x 1.75 Gy) thus supporting that a low leukemogenic retrovirus and a subleukemogenic irradiation may cooperate in the genesis of the radio-induced pathology.

If the new retrovirus encountered in primary radio-induced tumors is implied in the leukemogenic process, it may act via the activation of an adjacent cellular gene. Such a mechanism was demonstrated for thymic lymphomas induced by Moloney and MCF viruses. To support such an hypothesis for the radiation-induced recombinant provirus we are presently cloning some of these proviruses together with the flanking sequences. Our purpose is to examine if the genomic organization of the provirus itself could be related to its tropism and pathogenic potential. We will also study the organization and expression of the cellular genes close to the integration site as compared to the normal counterparts.

IV. Objectives for the next reporting period:

We reported the clonal integration of new recombinant ecotropic retroviruses in radio-induced primary tumors. During the next reporting period we will examine the role of such viruses in the neoplastic process. Firstly, we will extend our data and search for new proviruses in radio-induced tumors of other mouse strain. Secondly, some new proviruses will be cloned (this work is presently in progress) and their genomic organization examined with respect to the cellular tropism and pathogenicity. The flanking sequences, after cloning, will be analysed with respect to modifications, amplification and expression in different tumors and compared to their normal counterpart.

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

Dept. Radiobiologie, CEN/SCK, Boeretang 200, B-2400 Mol, Belgium.

Abteilung für Pathologie der Gesellschaft dür Strahlen und Umwelftforschund, Neuherberg, Germany.

VI. Publications:

1987

J. GHYSDAEL, C. BRUCK, R. MAMOUN and A. BURNY -
Genetic structure of bovine leukemia virus genome and biosynthesis of bovine leukemia virus proteins.
A. Burny and M. Mammerickx (eds.), Enzootic Bovine Leukosis and Bovine Leukemia Virus. Martinus Nijhoff Publishing, Boston, 1987.

H. BAYLAC-KALABOKIAS, T. ASTIER-GIN, E. LEGRAND, J.F. DUPLAN and B. GUILLEMAIN -
Evidence of recombinant ecotropic provirus integration in thymic lymphomas induced by direct or indirect radiation effects. (soumis)

D. VAILLIER, R. DACULSI, E. LEGRAND and B. GUILLEMAIN -
Cytokines released by lipopolysaccharide stimulated spleen cells from lymphoma grafted AKR mice. (soumis)

J.P. MERLIO, E. LEGRAND, O. GARRAUD, D. VAILLIER, A. De MASCAREL, B. HOERNI and B. GUILLEMAIN -
Natural killer activity in peripheral blood of patients with malignant lymphomas. Correlation with histological grade. (soumis)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 2636

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 combined application of different radionuclides, paying particular attention to the effects of different qualities of radiation, radiation dose rates and time patterns. The potential synergistic effects at very low irradiation doses are of special interest.

II. Objectives for the reporting period:

a) The effect of dose protraction with a very low activity of ^{224}Ra (18.5 kBq/kg).

b) Osteosarcoma induction with a relatively low activity of ^{227}Th incorporated short before the age range of spontaneous osteosarcoma.

c) Effects of multiple injections of colloidal radionuclides for local osteosarcoma induction.

d) Labelling of mineral metabolism of bone tumour grafts with ^{47}Ca and ^{224}Ra.

III. Progress achieved:

1. Methodology

Parts a - c are long-term studies into the pathology of late effects

a) Female NMRI mice received 18.5 kBq/kg ^{224}Ra (alpha-emitter, half-life 3.64 days), corresponding to 0.15 Gy mean skeletal dose, either as a single i.p. injection at age of 1 month or as 72 injections (2 per week) of 257 Bq/kg through 36 weeks, starting at age of 1 month.

b) Female NMRI mice received i.p. 37 kBq/kg ^{227}Th (alpha-emitter, half-life 18.7 days), corresponding to 2 Gy mean skeletal dose, at age of 1 month or 18 months.

c) Colloidal solutions of radionuclides were injected locally at the tibia of 10-week-old female NMRI mice.

d) Four transplantation lines from radiation-induced osteosarcomas were investigated. They were maintained by continuous transplantation (i.m. injection of tumour tissue) over a number of years. The tracer experiments were carried out with a double-labelling of ^{47}Ca plus ^{224}Ra. The nuclides were determined separately and their concentrations measured in skeleton, liver, kidney, spleen, muscle, and in the tumour tissue. The concentration in the latter was assessed using the concentration in muscle tissue from the thigh as a reference.

2. Results

a) TABLE 1 Osteosarcoma rate (i.e. frequency of cases per 100 day interval as related to surviving animals) after incorporation of 18.5 kBq/kg ^{224}Ra (female NMRI mice). Effect of dose protraction.[1)]

time interval*	Injection schedule: single injection		72 injections[1)] (2 per week) through 36 weeks	
101 - 200	0 %	(0/295)	0 %	(0/296)
201 - 300	1.0 %	(3/295)	0.4 %	(1/282)
301 - 400	0.3 %	(1/290)	0.8 %	(2/249)
401 - 500	0.7 %	(2/284)	1.2 %	(3/241)
501 - 600	1.1 %	(3/262)	0.9 %	(2/219)
601 - 700	0.5 %	(1/224)	0.5 %	(1/189)
701 - 800	4.7 %	(7/150)	3.7 %	(5/135)
801 - 900	0 %	(0/78) [2)]	5.4 %	(4/74) [2)]
901 - 1000	0 %	(0/28) [2)]	4.8 %	(1/21) [2)]
1001 -	0 %	(0/5) [2)]	33.3 %	(2/6) [2)]

* days after start of experiment at 4 weeks of age
1) In the group with dose protraction 13 % of the animals developed malignant lymphoblastic lymphoma during the first 300 days of the experiment.
2) p = 0.005 for the difference of frequency after 800 days of the experiment

b) TABLE 2 Osteosarcoma induction after incorporation of 37 kBq/kg ^{227}Th at different ages, female NMRI mice

Age at injection	Incidence: absolute	cumulative[3)]	Time after incorporation (days): first tumour	average ± s
1 month	21 % (9/ 43) [1)]	32 %	344	613 ± 174 [4)]
12 months	16 % (19/122) [2)]	43 %	274	391 ± 91
18 months	12 % (11/ 96)	36 %	71	316 ± 98 [4)]

1) Value from a previous experiment (report 1984) since in the parallel running group only one osteosarcoma has been observed at time of evaluation.
2) Value from a previous experiment (report 1984) for comparison
3) Corrected for competing risk (up to ten surviving animals)
4) Logrank-test p < 0.01

c) TABLE 3 Local osteosarcoma induction
Start of experiment: March 86 Present status: Dec. 1st, 1987

Nuclide	1st inj. dose kBq per animal [total number of mice]	2nd injection (6 months) (half of the mice)			2nd injection (9 months) (half of the mice)		
		dose kBq per animal	actual death rate %	actual bone tumour rate, %	dose kBq per animal	actual death-rate %	actual bone tumour rate, %
^{141}Ce	40 - 70 [58]	21.7	37.9	--	91.2	43.3	3.3 (one case)
^{177}Lu	394 [59]	374	46.7	--	681	33.3	--

	dose kBq/animal	total number of mice	bone tumour rate number	bone tumour rate %	
^{144}Ce	20 - 37	49	41	84	last mouse died 726 days after start of experiment

TABLE 4 Local injection of ^{227}Th (half-life 18.7 d)
Start of experiment: Dec. 4th, 1985

group	I	II	III
inj. activity (kBq)	3.7	12.3	37
number of animals	20	20	20
local osteosarcomas	0	0	0
systemic osteosarcomas	5	12	1
survivors	as at Dec. 1st, 1987		
	2	0	0
mean life-time (d)	580.1	394.3	313.4*
s %	19.9	24.6	12.9

*radiotoxic effect

d) The Ca-concentrations in the tumour tissue were only slightly higher than those in muscle, whereas the Ra-concentrations were distinctly higher (up to factor of ten) in the tumour tissue, in particular in two of the graft types.

3. Discussion

a) Dose protraction of an activity of ^{224}Ra as low as 18.5 kBq/kg resulted in changes of the target tissue of oncogenesis (i.e. in addition to osteosarcoma induction of malignant lymphoma) and prolongation of the osteosarcoma risk until to the end of the life-span.

b) Incorporation of 37 kBq/kg ^{227}Th at age of 18 months in female NMRI mice the osteosarcoma risk is the same as in younger animals, since at older age the latency period of radiation-induced osteosarcoma is shortened.

c) For the induction of local osteosarcomas a longer continuous irradiation period seems to be essentially necessary for beta-irradiation as well as most probably for alpha-irradiation too.

d) The reported results which shall be completed by autoradiographic methods indicate that the transplanted bone tumour tissue did not "forget" completely its osteogenic origin.

IV. Objectives for the next reporting period:

a) Evaluation of non-bone tumour frequency especially malignant lymphoma/leukemia in experiments with short-lived bone-seeking radionuclides. Influence of dose-time pattern, radiation quality and 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) Use of an incorporated ^{228}Th-deposit (alpha-emitter, 2 years half-life), which may produce local osteosarcomas at the injection site by continuous irradiation and which moreover releases ^{224}Ra into the circulation continuously. This method would allow us to study the effects of protracted ^{224}Ra in any desirable dose range. Using very low alpha-doses it could serve as a model for the plutonium risk.

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

VI. Publications:

Gössner, W., Luz, A., Murray, A. B.: The role of pathology in late effect studies. In: Proceedings of the 8th International Congress of Radiation Research, Vol. 2 (Eds.: E. M. Fielden et al.). London, New York, Philadelphia: Taylor & Francis, 658-663 (1987)

Müller, W. A.: Age related retention and dose burden after injection of ^{224}Ra and ^{227}Th in mice. In: Age-related factors in radionuclide metabolism and dosimetry (Eds.: G. B. Gerber et al.). Dordrecht, Boston, Lancaster: Martinus Nijhoff Publishers, 145-148 (1987)

Title of the project no.: 2

Pathogenesis of radiation-induced cancer

Head(s) of project:

W. Gössner

Scientific staff:

V. Erfle, J. Schmidt, M. Casser-Bette, 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) Characterization of c-fos and c-myc protooncogene expression in the cartilage organ culture system of the mandibular condyle from neonatal mice during the early phase of osteogenic differentiation in vitro.
b) Determination of the critical sequences of osteosarcoma-inducing MuLV genomes with respect to tissue specificity and pathological potential.
c) Effect of a macrophage-stimulating alkyl-lysophospholipid administered during the progression period of radiation-induced osteosarcoma.

III. Progress achieved:

1. Methodology

a) Slot blot analysis was carried out with RNA isolated from mouse mandibular condyles cultured in the presence and absence of fetal bovine serum (FBS) for a period of 9 days. Specific c-fos and c-myc probes were used to determine the effect of FBS and mechnical forces on oncogene expression at various intervals after start of in vitro cultivation.

b) Molecular cloning of endpoint-diluted virus, restriction enzyme analysis, subcloning and sequencing of viral LTRs. Construction of CAT-vectors and CAT-assay.

c) 130 female NMRI mice received 6 i.p. injections of 37 kBq/kg ^{227}Th (time interval 2 weeks) starting at 4 weeks of age. 80 of these animals and 80 not ^{227}Th-treated mice received the alkyl-lysophospholipid ET-18-OCH_3 orally (10 or 20 mg/kg respectively on 6 days per week) starting at day 201 of the experiment until day 772 (i.e. day 800 of life and until to the end of life-span of ^{227}Th-contaminated mice). There were 50 untreated controls.

2. Results

a) Uninfected control condyles cultured in the presence and absence of FBS showed a high transient expression of the c-fos protooncogene which peaked at 0.5 hours after start of the culture, decreased thereafter until 6 hours and was undetectable for the rest of the 9 day culture period. Addition of FBS at 6 or 18 hours after start of the culture did not induce c-fos expression. In contrast, mechanical forces applied to the tissue at 6 hours of tne culture period induced a high transient c-fos expression which was again down-regulated within 4 hours. Low c-myc expression was detected throughout the culture period.

b) The genomes of the RFB and OS-5 strain of murine leukemia virus (MuLV) were molecularly cloned and characterized by the RNA fingerprint technique and restriction enzyme

analysis. Both retroviruses are highly related to AKV MuLV. The RFB virus contains a variant envelope region which differs from that of AKV and OS-5. Comparative sequence analysis of MuLV LTRs revealed rearrangements of the binding sites for nuclear factors. The activity of the retroviral LTRs in CAT-assays could be stimulated by glucocorticoid hormone in fibroblasts and differentiated osteogenic cells.

c) The cumulative osteosarcoma incidence corrected for competing risk and calculated until less than 10 animals survived was not significantly influenced by the additional ET-18-OCH_3 (E) treatment: ^{227}Th alone 73 %, ^{227}Th + 10 mg/kg E 65 %, ^{227}Th + 20 mg/kg E 63 %. One case of osteosarcoma was observed in the 130 animals without ^{227}Th incorporation.

3. Discussion

a) The high transient expression of c-fos in mandibular condyle tissue is independent from factors present in FBS. Rather, mechanical forces applied to the tissue during the preparation procedure or experimentally at 6 hours after start of the culture induce c-fos expression, which is down-regulated to undetectable levels 4 hours later.

b) The activity of the LTRs of bone tissue-derived retroviruses depends on the structure of the enhancer region and the state of differentiation of the cells. It can be regulated by glucocorticoid hormone.

c) The lacking influence of orally administered alkyl-lysophospholipid on the development of radiation-induced osteosarcoma corresponds with the low antigenicity of this tumour as described by Nilsson.

IV. Objectives for the next reporting period:

a) Further studies on the expression of c-fos, c-myc and v-fos oncogenes in mouse mandibular condyles after transformation and induction of a osteosarcoma-like osseous lesion by FBR osteosarcoma virus.

b) Further studies on the tissue specific activation of retroviral LTRs.

c) Final evaluation of an experiment studying the effect of Cyclosporine on ^{227}Th-induced oncogenesis.

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

Department of Molecular Biology, University of Aarhus, DK-8000 Aarhus, Denmark (Dr. F. S. Pedersen)

Department of Molecular Biology, C.E.N./S.C.K., 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., Casser-Bette, M., Murray, A.B., Luz, A., and Erfle, V.
Retrovirus-induced osteopetrosis in mice. Effects of viral infection on osteogenic differentiation in skeletoblast cell cultures.
Am. J. Pathol. 129, 503-510 (1987)

Schmidt, J., Casser-Bette, M., Rodan, G.A., Baumgartner-Decker, C., and Erfle, V.
Bone tumor-inducing retroviruses and osteoblastic cells in vitro: Target cell specificity and effects of viral infection.
In: Current Advances in Skeletogenesis III (Eds.: S. Hurwitz, J. Sela). Plenum Press, 73-91 (1987)

Silbermann, M., Schmidt, J., Livne, E., von der Mark, K., and Erfle, V.
In vitro induction of osteosarcomalike lesion by transformation of differentiating skeletal precursor cells with FBR murine osteosarcoma virus.
Calcif. Tissue Int. 41, 208-217 (1987)

Silbermann, M., Schmidt, J., Livne, E., von der Mark, K., and Erfle, V.
Induction of in vitro osteosarcoma by transformation of differentiating skeletal precursor cells with FBR murine osteosarcoma Virus (FBR MuSV).
In: Current Advances in Skeletogenesis III (Eds.: S. Hurwitz, J. Sela). Plenum Press, 100-107 (1987)

Melchiori, A., Allavena, G., Böhm, J., Remy, W., Schmidt, J., Parodi, S., Santi, L., and Albini, A.
Interferons inhibit chemotaxis of transformed cells and their invasion of a reconstituted basement membrane.
Anticancer Res. 7, 475-480 (1987)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

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

Contract no.: BI6-D-083-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 2636

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. 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 Radium-224.

Project 1 is concerned with more than 1500 ankylosing spondylitis patients treated between 1948 and 1975 with repeated intravenous injections of Radium-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 Radium-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 Radium-224 treatment.

III. Progress achieved:

In this epidemiological study of the somatic late effects risk after incorporation of a short-lived alpha-emitter we are following 1571 ankylosing spondylitis patients. These patients have been treated for their disease with repeated intravenous injections of Radium-224. The usual injection scheme which is applied even nowadays consisted of a total of 10 - 12 injections of about 1 MBq each, given at weekly intervals.

The resulting dose to the skeleton is 0.56 to 0.67 Gy for a 70 kg man. The skeletal doses in this project are much lower than in project 2.

In addition, there exists a control group of ankylosing spondylitis patients not treated with Radium-224 in order to provide comparative information on causes of death and lesions possibly related to the basic disease itself or to chemotherapeutics. Current follow-up has shown that a substantial part of them has been treated with X-rays previously. These patients are likely to be deleted from the control group after completion of the current course of follow-up.

So far, 505 patients in the exposure group and 642 patients in the control group, have died (Table 1). Causes of death have been ascertained in 493 patients in the exposure group and in 524 patients in the control group.

The skeletal and soft tissue diseases observed so far are listed in Table 2. For the incidences of kidney and liver diseases, as well as for cataracts, there is no striking difference between exposure and control group.

Diseases and haematopoietic tissue among living and dead patients include: bone marrow failure (11 in the exposure group vs. 8 in the control group) and leukaemias (7 cases vs. 6). In the control group most of the leukaemias were of the acute lymphoblastic type and there was no chronic myeloid leukaemia even though 3 cases of the latter were observed in the exposure group.

Myeloproliferative diseases, mostly acute or chronic leukaemias, have been reported following Thorotrast application too: 10 cases were observed among 432 deaths in a group of 878 well examined Thorotrast patients followed in the Federal Republic of Germany. This relatively high incidence of myeloid leukaemias in the Thorotrast patients seems very surprising because of the very low dose rate of only 1.2 mGy per week of alpha skeletal dose. On the other hand, much higher doses, but also at higher dose rates produced no cases of leukaemia in the juveniles of project 2. The dose rates, as well as the incidence of myeloid leukaemia, for both, the patients in this project, and the adults in project 2 is in between.

Although the number of cases is low for the groups at the higher dose rates one should consider that not only the total dose but also the dose rate may play an important role in the induction of malignancies in man. In animals, rather low dose rates of bone seeking alpha-emitters have been demonstrated by different authors to induce leukaemias.

Table 1: Follow-up status of ankylosing spondylitis patients in the exposure and control groups (Dec. 1987)

	Exposure group	Control group
Total number of patients	1571	1535
Patients contacted in 1987	22	186
Deceased patients	505	642
Death cause ascertained	493	524

Table 2: Skeletal and soft tissue diseases (Dec. 1987)

	Exposure group	Control group
Malignant skeletal tumours	3	0
Exostosis	0 (+ 1L*)	1
Bone marrow failure	6 (+ 5L)	5 (+ 3L)
Leukaemias	5 (+ 2L)	6
Acute leukaemias	1	6
Acute lymphoblastic leukaemias	1	4
Chronic leukaemias	4 (+ 1L)	0
Chronic myeloid leukaemias	3	0
Total cancers	79 (+16L)	110 (+15L)
Kidney diseases	55 (+80L)	60 (+57L)
Liver diseases	21 (+54L)	30 (+23L)
Cataracts	4 (+20L)	1 (+14L)

*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 risk in bone, haematopoietic tissue, kidney, liver, and other organs known or supposed from project 2 to be affected by injected Radium-224.

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

10 Orthopaedic and Rheumatic hospitals in Western Germany and Berlin.

Close cooperation with the working groups in projects 2 and 3 of this contract.

VI. Publications:

WICK, R. R.:
Ergebnisse einer Follow-up Studie ^{224}Ra-behandelter Bechterew-Patienten.
Akt. Rheumatol. 12, 33-37 (1987)

WICK, R. R., GÖSSNER, W.:
^{224}Ra in man: Long term effects on bone and haematopoietic tissue.
8th International Congress of Radiation Research, Edinburgh (UK),
July 19-24, 1987
Abstract in: Proceedings, Vol. 1, p. 210

Addendum for 1986:

RABENSEIFNER, L., WICK, R. R.:
Spätergebnisse nach Radium 224-Behandlung bei ankylosierender Spondylitis.
Akt. Rheumatol. 11, 223-226 (1986)

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 Radium-224 as juveniles and adults.
Determination of stochastic and non-stochastic radiation effects and their dose, time, and age dependence.

II. Objectives for the reporting period:

Review of existing data.

Evaluation of diagnosis of kidney damages.

New questionnaire sent to adults - first interpretations in collaboration with Prof. F. Stefani (Eye Hospital of the University of Munich).

Cooperation with Prof. C. W. Mays (National Cancer Institute/NIH, Radiation Epidemiology Branch, Bethesda/MD, USA) in the cataract and kidney study.

III. Progress achieved:

In this study we followed the health of 900 persons who received repeated injections of Radium-224 about 35 years ago, mostly for treatment of tuberculosis and ankylosing spondylitis.

From the questionnaire sent to patients during the last reporting periods we found an increased incidence of cataracts. Until December 1987 eighteen cases acc. to about 9 % were reported in juveniles and 47 cases acc. to 6.5 % in adults. In cooperation with Prof. Stefani we started a program which included a questionnaire sent to ophthalmologists and screening of persons below 55 years of age.

Concerning the kidney examinations we will continue our follow-up of last year. Until now the response rate of the diagnosis reports sent to the patients is only about 50 %. These results include three new kidney diseases, three borderline cases, and one hypernephroma.

In our screening for deceased patients we found one new case of chronic lymphatic leukaemia. This is the only case in the group of juveniles appearing 38 years after injection of Radium-224.

For 1988 we will continue our follow-up including examinations for possible diseases in the second generation.

Prof. Mays has been invited for August 1988 to compare our data and for helping the preparation of papers for a symposium in Bethesda/MD.

Table 1: Summary of the Radium-224 patients in project 2 (Dec. 1987)

	Age at first injection 1-20 yr	Adult	Total
Traced patients	218	682	900
Deaths	90	419	509
New deaths since 1986	5	26	31
Skeletal diseases:			
Bone sarcoma	37	18	55
Exostosis	29	0	29
Growth retardation	28	0	28
Tooth breakage	40	20	60
Soft tissue diseases:			
Cancer of soft tissue	14	66	80
Leukaemia	1	5	6
Kidney diseases	11	79	90
Liver diseases	4	36	40
Cataract	18	47	65

Table 2: New malignant soft tissue diseases

Pancreas carcinoma	0	2	2
Bronchial carcinoma	0	1	1
Rectal carcinoma	0	1	1
Chronic lymphatic leukaemia	1	0	1

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.

Preparing papers for a symposium in Bethesda/MD.

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:

CHMELEVSKY, D., KELLERER, A. M., LAND, C. E., MAYS, C. W., SPIESS, H.: Time and dose dependence of bone-sarcomas in patients injected with Radium-224.
Radiat. Envr. Biophysics (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. Depner, H. Friede

I. Objectives of the project:

The project is aimed 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 in particularly important in view of the new risk estimates emerging after the revision of the Japanese dosimetry and also in view of the increased interest, after the reactor accident, in the problem of low doses of ionizing radiations.

II. Objectives for the reporting period:

There were four major objectives for the reporting period. The first was the continued work on mathematical methods for the analysis of radiation carcinogenesis studies. The second was the effort to utilize the new results from RERF after the termination of the dosimetry revision; this was linked with attempts to contribute towards the initiation of follow-up studies planned by the Soviet authorities after the reactor accident. The third was the continued collaboration with experimental groups in CEN, Fontenay-aux-Roses on the effects of radon daughters and of different densely and sparsely ionizing radiations. The fourth aspect is the continued close cooperation with the Radium-224 follow-up studies.

III. Progress achieved:

1. Mathematical methods for the analysis of radiation carcinogenesis

A number of algorithms for optimization problems have been developed which are required in maximum likelihood fits to analytical models of the dose, time, and age dependence of tumour rates. These algorithms were sufficiently simple to be written for microcomputers. The utilization of the powerful, but large optimization program GRGA (developed by J. Abadie) has become so essential for the current studies, that it was decided to implement this program, too, for use on microcomputers. Although this required various changes, the implementation has now been successfully completed. Being now far more readily applicable, the program has become a main tool in a variety of modifications of the Cox proportional hazards algorithm in non-parametric and in partly parametric form.

2. Studies of low dose radiation risks after the revision of the Japanese dosimetry

In last year's report investigations were described which were based on the data on cancer mortality up to 1982, now available from RERF. These studies have, in the meantime, been extended with particular emphasis on an assessment of the changes of risk coefficients resulting from the continuation of the observations to 1985 and from the dosimetry revision. The first phase of this work consisted in a utilization of population statistics data, largely from the US SEER-tables, to derive essential relations on the time dependence of tumour mortality in the two sexes, and of the implication of these relations for the distribution in age of detriment from radiation exposures at different ages. In this way it has become possible to determine, for solid tumours, the fraction of detriment expressed in specified periods of the follow-up of the atomic bomb survivors. It is seen that the revised dosimetry contributes little to the increase of the risk estimates for solid tumours, and that the main increase is due to the continued observation since 1975, when ICRP published its previous risk estimates. The continuation caused an increase of the risk estimates by about a factor 2. A further increase by a factor 2 can arise, if the postulates of the relative risk model continue to apply in the forthcoming decades. For leukaemias the situation is different, because the increase of the risk estimates is, indeed, due to the revised dosimetry. It is also seen that the validity of an absolute risk model for leukaemias need not necessarily exclude an added relative risk component for old age leukaemias, especially for those exposed at young ages.

The work on the further analysis of the Japanese data has been linked with activities which were sponsored by IAEA and WHO to prepare plans, jointly with Soviet colleagues for an epidemiological follow-up of those who were evacuated from the surrrounding of Chernobyl (WHO, Environmental Report 25, 1987).

3. Cooperation with CEN, Fontenay-aux-Roses, on the effects of radon daughters, neutrons, and gamma-rays

Earlier cooperation with CEN, Fontenay-aux-Roses has been concerned with the comparative effectiveness of radon daughters and neutrons to induce lung cancers in Sprague-Dawley rats. The recent utilization of solid state detectors in the radon exposure facilities has led to the recognition that earlier values assumed for the radon daughter exposures were substantially too large in some experiments. Additional radon exposures were therefore performed with improved dosimetry, and the collaboration with CEN was concerned with the derivation of new equivalence ratios for radon, neutrons, and gamma-rays. The equivalence ratio of radon to neutrons has been found to be about a factor of 2 higher than earlier assumed. The relative biological effectiveness of fission neutrons relative to cobalt gamma-rays for lung cancer induction in the Sprague-Dawley rats is consistent with the high RBE values obtained at low doses in other systems and it supports the proposed revision of the quality factors for neutrons. The analysis has been performed in terms of maximum likelihood procedures utilizing the accelerated hazard-function model and a shifted time model which led to similar results. The work was done in cooperation with the Institut für Strahlenschutz of the GSF, Neuherberg, where the greater part of the numerical analyses was performed.

4. Cooperation with the Radium-224 follow-up studies

The work on the analysis of the cataracts in the German Radium-224 patients has been continued, and this collaboration will be extended in view of the new program of clinical examinations which has now been initiated.
A new mathematical analysis has been performed for the osteosarcomas in the patients of project 2, to link our earlier non-parametric analysis to the parametric analysis which is utilized in the NIH radio-epidemiological tables, and in the work of Land. This study, jointly performed with Drs. Chmelevsky, Land, Mays, and Spiess, has substantiated the temporal distribution which is adequately represented by a logarithmic normal distribution of appearance times of the osteosarcomas. But it has also shown that, in contrast to the values adopted for the radio-epidemiological tables and in agreement with our earlier non-parametric analysis, the risk coefficients at low doses are smaller by about a factor 2.

IV. Objectives for the next reporting period:

The work on the Japanese data will assume increasing importance, as new data come out of RERF. The studies will be performed in close cooperation with colleagues from the United States, and there will also be a special effort to cooperate with Czechoslovakian colleagues, as their data on the uranium miners are particularly relevant to the question, whether the radiation-induced relative risks persist throughout life.

A problem that requires further study is the assumed dose rate dependence of osteosarcomas in the Radium-224 patients.

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

VI. Publications:

KELLERER, A. M.:
Models of cellular radiation action
In: Kinetics of Nonhomogeneous Processes (Ed.: G. R. Freeman).
New York: J. Wiley & Sons, 305-375 (1987)

KELLERER, A. M.:
Zur Revision der Qualitätsfaktoren im Strahlenschutz.
In: Strahlenschutz nach Tschernobyl (Hrsg.: J. Schütz u. a.).
Stuttgart, New York: G. Thieme, 20-34 (1987)

KELLERER, A. M.:
Microdosimetry - Recent trends and applications to radiation biology and radiation chemistry.
In: Proceedings 8th International Congress of Radiation Research, Vol. 2 (Eds.: E. M. Fielden et al.). London, New York, Philadelphia: Taylor & Francis, 338-344 (1987)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 2636

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. A.M. Kellerer and the "Kinderpoliklinik der Universität München", Prof. Dr. H. Spiess.

Title of the project no.: 1

Epidemiological Investigation on Radiation Cataracts by the Ophthalmological Examination of Patients Who had Received Ra-224

Head(s) of project:

Prof.Dr.Heinz Spiess

Scientific staff:

Prof.Dr.Fritz Stefani, Karl Kogler

I. Objectives of the project:

The project is designed to supplement an earlier study of cataracts in radium-224 patients which was performed within the framework of research contract BI-6-0083-D (B). In this study which is now being published cataract rates were analysed by epidemiological methods, i.e. mostly without systematic serial ophthalmological examinations of the patients. Such examinations will now be performed for those patients who were children or juveniles when injected with radium-224 and are now still under 55 years of age.

The serial examinations provide 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 (one year approximately) of the same patients to assess the evolution of the radiation induced cataracts. The aim is to gain added insight into the time and dose dependences of the radium-224 induced cataracts.

II. Objectives for the reporting period:

The main initial objective was to contact the patients who were injected as children or juveniles and who are now still below 50 years of age. For the patients with whom the contact had been kept, detailed questionaires had to be designed and to be sent to their eye doctors. The patients had to be invited to visits at the Munich University Eye Clinic for examinations by Prof.Stefani.

A possibility had to be created to utilize a Scheinpflug Camera, and since the necessary investement for such a camera exceeds the possibilities of this project, this had to be arranged with the Department for Experimental Ophthalmology at the University Eye Clinic in Bonn (Head of Department: Prof.Dr.Hockwin). The Scheinpflug Camera will make it possible to observe radiation induced cataracts even in the presence of inchoate spontaneous cataracts.

III. Progress achieved:

The detailed questionnaire has been designed by Prof.Stefani and has been distributed to the eye doctors of the patients. As a result the examinations by the patients' own eye doctors and their answers have gained sub stantially in consistency and comparability.

129 patients were defined who are especially eligible for the new program. 45 of the ophthalmologists of these patients have already answered the questionaires, and 9 patients have been examined at the Munich University Eye Clinic by Prof.Stefani. In the past year Prof.Stefani was able to recognize two early cataracts which had all the characteristics of being radiation induced. At present the number of cataracts among patients injected as children or juveniles is 18.

Final arrangements have been made to use the Scheinpflug Camera at the Eye Clinic of the University of Bonn.

IV. Objectives for the next reporting period:

Collection of more diagnoses from the ophthalmologists of the radium-224 patients.
Screening of cataract patients at the Eye Hospital of the University of Munich by Prof.Stefani.
Invitation of added patients with no diagnosed cataract for examination.

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

Dr.Danielle Chmelevsky, GSF, Neuherberg, Institute for Radiation Protection.
Prof.C.W.Mays, Radiation Epidemiology Branch, National Cancer Institute, Bethesda/MD, USA.

VI. Publications:

Stefani, F.H., Spiess, H., Cataract in Ra-224 Injected Patients (in preparation).

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. (accepted for publication)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 - C65 - 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 %)
Dr. Horst Backhaus	(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 nuclectide 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:

a) INDUCED TRANSPOSITION OF TY ELEMENTS IN YEAST

1. Methodology: The methods detecting mutation induced by Ty insertion into the promotor region of ADH2 and ADH4 genes were described before. Briefly, antimycin A resistance and Southern hybridisation were used for detection of the frequency of actual transpositions among the mutants.

2. Results: The experiments to determine the dose effect relation of transposition after exposure with various agents show that there is a maximum at doses resulting in 10 - 30 % survival. At higher doses the fraction of mutants with Ty-insertion is much lower.

Transposition in into chromosomal DNA: A diploid tester strain showed a very low mutant yield with all agents tested. The spontaneous transposition frequency in this strain is normal.

A strain with a defective DNA repair gene (phenotype UV hypersensitive) showed a higher mutant yield after gamma irradiation and also a higher transposition number when compared with a normal tester strain. Spores of this strain obtained after a cross with a wild type strain were also tested. The UV repair proficient spores show a normal mutant yield but a reduced transposition number. One of the spore clones gave only one detectable transposition among 150 antimycin A resistant mutants. Normally 1/4 to 1/2 of the mutants have an insertion in the loci analysed.

Transposition of Ty elements into plasmids: In vitro mutations were performed to generate a strain with disruptions in the ADH2 and ADH4 genes, leading to 1 % of the spontaneous antimycin A resistant mutant frequency. In this strain the fraction of Ty integrants into a plasmid is 10% of all antimycin A resistant mutants. (see Figure)

Beside several newly integrated Ty elements we found two new genetic mechanisms leading to the resistant phenotype: (1) Four plasmids carried complex rearrangements resembling duplications of the ADH2 or ADH4 gene. (2) Four plasmids carried an integration of a 330 base pair element which was found to be highly repetitive in a normal yeast strain.

DNA sequence analysis was done, identifying the Ty integration site in the ADH2 coding region and into the upstream region of an ADH2 gene with a 500 base pairs deletion. Both integrations have taken place at areas where until now no Ty integration could be achieved. First analysis of the DNA sequence of one of the repetetive elements show a sequnece that is homologous to a delta element.

3. Discussion: The frequency of Ty transposition seems to be a genetically determined multifactorial process. The inducibility of transposition by mutagenic agents, however, is consistent in all the strains tested (except for the strain that did not transpose at all). Cenetic manipulations in the tester strains resulted in the possibility to detect further Ty insertions as well as complex rearrangements.

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: In previous studies we observed a definite decrease in transformation rate as dose-rate of gamma-rays was decreased from 1 Gy/min to 0.1 Gy/min and further to 0.02 Gy/min. The dose response curve which is upwards bending, consistent with a linar-quadratic or a quadratic function, becomes more straight as dose-rate is decreased. In order to study much lower dose rates methods were developed to irradiate the plated cells in the incubator continuously for up to 3 days. Dose rates of gamma-rays were 2 Gy/day and 4 Gy/d. In 10 experiments each consisting of 400 culture vessels a further decrease in transformation rate compared to 0.02 Gy/min (i.e. 28.8 Gy/d) was observed, although results were not significantly different between 2 Gy/d and 4 Gy/day, amounting roughly to a factor of 2.

However, this further decrease may be due to a decreased sensitivity to transformation as cells proliferate after plating. This was confirmed by 2 pilot experiments where plated cells were irradiated with a delay of 2 days at a dose rate of 1 Gy/min yielding a transformation frequency which was only marginally higher than that by continuous irradiation for 2 days.

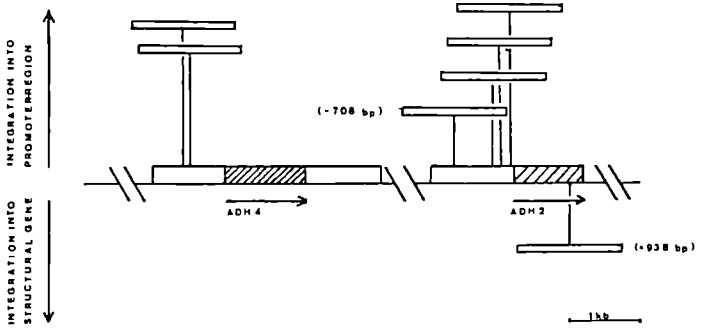


Figure: Cloned fragments are shown as thick bars, structural genes as hatched areas. The Ty-elements are shown as thin bars and are not drawn to scale. The direction of transcription is indicated by arrows. All Ty-integrants isolated are transcribed in the opposite direction with respect to the structural genes. Where known numbers in parenthesis show the integration point with respect to the adenin of the start codon. All other integration sites are determined within a range of approximatly 150 bp.

IV. Objectives for the next reporting period:

a) Radiation induced transposition of mobile gene elements: Importance of recombination for transpositions events, effect of metabolic inhibitors, influence of DNA repair processes for induced transposition. Locus- and sequence specificity of transposition.

b) In vitro transformation of mammalian cells: Experiments on the importance of repair, change in sensitivity due to cell proliferation or due to decreased proliferative future at low dose rate irradiation.

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, Pijswijk, Netherlands

Dr. V. M. Willioamson, 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-Stuewer: Effect of irradiation and mutagenic chemicals on the generation of ADH2-constitutive mutants in yeast. Significance for the inducibility of Ty transposition. Mutation Research 177, 53-60 (1987)

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, submitted.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.
University of Aarhus
C.F. Møllers Allé 130
DK - 8000 Aarhus C

Telephone number: 06-125177

Title of the research contract:

Characterization of somatic mutations during radiation inouced 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, P.Jørgensen, K.L.Johansen, N.O.Kjeldgaard, S.Lovmand, H.S.Olsen, K.Paludan, F.S.Pedersen, L.Pedersen

I. Objectives of the project:

1) To identify genetic changes in proviral genes and other cellular genes 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.

One section of the research concerns the structure and function of murine leukemia viruses associated with osteoid or lymphoid tumours. We have previously detected highly variable repeat segment structures in the U3 part of the LTR region in these viruses. Evidence from other systems raises the possibility that these repeat segments have an effect on both the transcriptional and tumorigenic specificities of the viruses. Presently, we exploit two strategies in the study of LTR function:

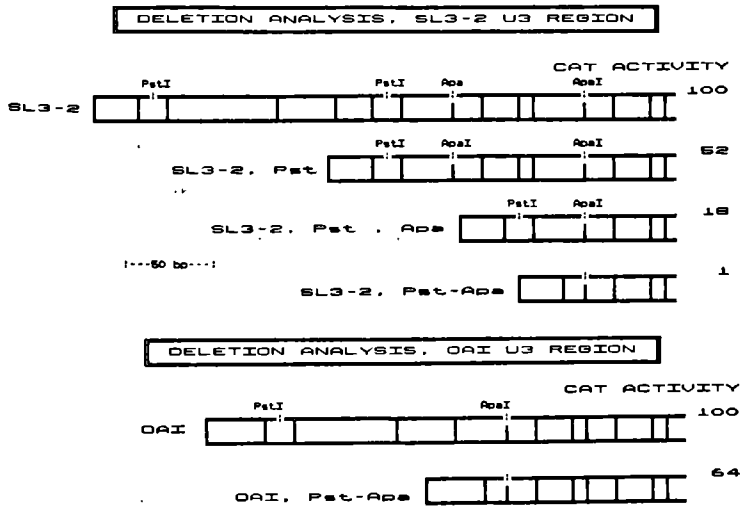
1) The interaction of nuclear proteins with dsDNA of the repeat segment region is analyzed. Our results indicate a complex set of interactions involving multiple proteins. To simplify the analysis we have used short synthetic DNA in our protein binding assays in conjunction with fractionation of nuclear protein extracts. Most efforts have been devoted to proteins in NIH 3T3 cells binding to the U3 region of Akv MuLV. One such protein is now being characterized after extensive purification.

2) The transcriptional activity of natural variant LTRs or mutagenized LTR elements is studied. For these studies two types of mammalian expression vectors have been constructed.

The first type is designed for introduction as naked vector DNA into cells in culture followed by determination of the transcriptional activity of the LTR in a transient expression assay. With this system a fine structure deletion mapping of the Akv U3 region and a rough mapping of the U3 regions of the lymphoid tumour derived SL3-2 virus and the osteoma derived OAI virus have been performed. Together with the protein binding analysis these results now serve to define functional elements in the LTR regions.

The second type of vectors permits transfer of foreign genes through steps of the normal retroviral life cycle. Although more time consuming this approach ensures stable insertion of one or a few copies of a well defined LTR directed transcription unit into the chromosomal DNA of the host cell. This method of gene transfer can be used with high efficiency for a large number of cell types, avoids non-physiological conditions, and can be used for both short and long time experiments. The methodology and assay conditions for this type of vectors have been established through studies of natural variant LTRs in a lymphoid cell line. We anticipate that our experience with this system will prove immediately valuable for studies of other cell types and of mutagenized LTRs.

In another part of our work we focus upon interactions between proviral and host DNA at the integration site. Such studies are of direct relevance for understanding of cases of retroviral tumorigenesis for which an effect of the integration site is possible. To facilitate such studies we have constructed a MuLV transmission vector system harbouring a procaryotic suppressor tRNA gene that permits selective recovery in Escherichia coli of proviral and cellular flanking sequences. The viability and stability of the modified viruses have been documented in cultured cells.
In collaboration with GSF/Neuherberg it has been found that injection of these viruses into newborn mice causes the development of different types of lymphoid tumours. The analysis of vector DNA sequences in tumour DNA is under way.



Transient expression activity in mouse fibroblasts cells of vectors carrying complete or deleted LTR regions as indicated. The expression levels were determined by enzymatic measurements of the product of the chloramphenicol acetyl transferase gene linked to the LTR.

IV. Objectives for the next reporting period:

1) To isolate and characterize additional viruses from bone tumours.

2) To perform detailled transcriptional analysis of additional viruses.

3) To analyze the cellular genes surrounding integrated proviruses in bone tumours.

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

The work is performed in close collaboration with the EULEP Task Groups nr. 1 on "Radiation induced osteosarcomagenesis" and Task group nr. 2 on "Radiation induced lymphomagenesis".

VI. Publications:

Pedersen, F.S., Etzerodt, M., Lovmand, S., Dai, H.Y., Bækgaard, A.J., Sørensen, J., Jørgensen, P., Kjeldgaard, N.O., Schmidt, J., Leib-Mosch, C., Luz, A. & Erfle, V.: Transcriptional control and oncogenicity of murine leukemia viruses. In: Viral Carcinogenesis, Alfred Benzon Symposium 24 (Kjeldgaard, N.O. & Forchhammer, J., eds.), Munksgaard pp 17-35, Copenhagen 1987.

Jensen, N.A., Jørgensen, P., Kjeldgaard, N.O. & Pedersen, F.S.: Expression of bovine papillomavirus type-1 late genes in cultured cells using retrovirus vectors. Cancer Cells 5, 131 - 135 (1987).

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, in press.

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 Pr68gag protein that is not myristilated. J. Virol. in press.

Strauss, P.G., Schmidt, J., Pedersen, L. & Erfle, V.: Amplification of endogenous proviral MuLV sequences in radiation-induced osteosarcomas. Int. J. Cancer, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Prof. P.H.M. Lohman
Labor. voor Stralen. & Chem. Mutag.
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 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.

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:

- Isolation of immortal SHE cell lines by the use of a selective and quantitative method
- Development of a focus assay for cell transformation with immortal SHE cells. Establishment of the number of steps involved after treatment with ENU
- Studies on the feasibility of complementation analysis via crosses with TOR- (thioguanine and ouabain resistant) mutants
- Characterization of normal, immortal and transformed SHE cells in structure of oncogenes and their rate of transcription

III. Progress achieved

- Isolation of immortal SHE cell lines by use of a selective and quantitative method

Methodology

Tertiary SHE cells were treated with B( )P for 24 hours. The treated cells were split into a large number of subpopulations which, after a period of 5 days in complete medium for expression of induced mutations, have been seeded in medium with low serum in which immortalized cells have a selective advantage. Cell populations which do not contain immortalized cells die out soon.

Results

The frequency of induction of immortalization, based on the number of viable cells after treatment was about 1.2×10^{-5}.

Discussion

The data suggest that immortalization occurred immediately after treatment. In some cell lines a second step occurred long after treatment, which improved the growth characteristics and cloning efficiency of the cell line, but these can be explained by spontaneous mutation. The assay appears to allow the determination of immortalization frequencies, although the assay is not as accurate as the assay for mutation induction.

- Development of a focus assay for cell transformation with immortal SHE cells. Establishment of the number of steps involved after treatment with ENU

Methodology

Confluent cultures of immortal cells were treated with ENU. AFter treatment cells were trypsinized and reseeded in petri dishes (P90) at a density of 2.5×10^5 cells in DMEM supplemented with 10% FCS. After 24 hrs this medium was replaced with medium with 1% serum which was refreshed every 3-4 days until foci of transformed cells were visible on the monolayer of contact inhibited immortal cells.

Results

The frequency of transformation increased linear with carcinogen concentration and was quantitatively similar to the frequency of ouabain resistant mutants, induced by the same carcinogen in the same cells. The transformation frequency was independent of the expression time (period of exponential growth between treatment and seeding for focus formation).

Discussion

The results are very similar to those obtained by us in an earlier study on ENU-induced focus formation in the immortal C3H10T½ mouse cell line. It remains to be established whether the observed transformation frequency in immortal SHE cells is also strongly influenced by cell density as is the case for C3H10T½ cells and whether transformation can be induced in the immortal SHE cells by X-rays.

- Studies on the feasibility of complementation analysis via crosses with TOR- (thioguanine and ouabain resistant) mutants

Methodology

Hybridization was induced by PEG-treatment under standard conditions.

Results

A TOR-mutant was isolated from the immortal BP_1 cell line in 2 successive steps (thioguanine resistance and ouabain resistance). These cells were crossed with mortal SHE cells, immortal ENU-1 and BP_1 cells.

Two pilot experiments showed that proliferating hybrids were obtained in all three crosses. The number of proliferating hybrids appears comparable

in the crosses of the immortal lines BP_{11} (TOR) x BP_1 and BP_1-(TOR) x ENU, while the number of proliferating hybrids was lower in the cross of immortal x mortal BP_1 x SHE.

Discussion

For definite conclusions elaborate progeny testing of the proliferating hybrids is required. A handicap might be that the isolation of TOR-mutants requires many cell generations, during which the cell line may undergo changes, which overgrow the original population. It has been observed that with increasing passage of BP_1 cells the frequency of anchorage independent (Aga^+) variants increases progressively (see next section). It was found that these Aga^+ variants have a higher growth rate than the original cells. Therefore it is envisaged to obtain neo^r and gpt^r cells for cell fusion via transfection which can be obtained in a much smaller number of cell generations.

- Comparison of normal, immortal and transformed SHE cells in structure of oncogenes and their rate of transcription

Methodology

Normal, immortal and ENU-induced transformed cells are compared in growth in agar, tumorigenicity and response to growth factors. In addition immortal BP_{11} cells were transfected with an activated ras gene.

Results

Transfection of BP_1 with a plasmid containing an activated ras gene (pEJ) resulted in transformed foci. The transformed cells were shown to contain integrated plasmid DNA and to express plasmid specific RNA.

Normal, immortal BP_1, ENU-transformed BP_1 and PEJ transformed BP_1 cells were compared in growth in agar. High cloning efficiency was observed for transformed cells, which immortal cells early after isolation did not grow in agar. Immortal BP_1 cells gave rise to some colonies in agar after prolonged culture. This correlated with tumorigenicity in nude mice: transformed cells gave rise to tumors, early immortal cells did not, while late immortal cells gave rise to tumors only after a long latency period. Further it was found that normal and immortal cells responded to the addition of external growth factors (PDGF and EGF) while an ENU-transformed line did not.

Discussion

Considering the sharp transitions from normal to immortal and from immortal (TP_1) to transformed this series of cell lines appears a suitable model to identify changes in expression of oncogenes which could be relevant to immortalization and transformation.

IV. Objectives for the next reporting period:

- Study of induction of immortality in SHE cells by X-rays
- Study of induction of transformation in immortal cells by X-rays. Determination of the effect of cell density
- Study on complementation analysis in immortality via crosses with transfected dominant genes and/or with TOR- (thioguanine and ouabain resistant) mutants
- Expression of proto-oncogenes in successive stages of transformation of SHE cells

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

Department of Medical Biochemistry, 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 enhances N-ethyl-N-nitrosourea-induced morphological transformation of Syrian hamster cells. Carcinogenesis (accepted for publication).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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. J.R. Maisin
Département de Radiobiologie
CEN/SCK
Boeretang 200
B - 2400 Mol

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

Mechanism of radiation-induced leukemogenesis.

Head(s) of project:

M. Janowski

Scientific staff:

B. Borremans
M. Janowski

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, namely those of the C57BL/Ka strain. We decided to use the radiation leukemia virus (RadLV), isolated from a radiation-induced thymic lymphoma of this strain, as a tool to investigate the molecular mechanisms by which it exerts its leukemogenic potential and to trace back the events leading to the appearance and development of thymic lymphomas upon irradiation.

II. Objectives for the reporting period:

1) At the end of 1986, we reported the presence of a novel RNA in radiation leukemia virus-induced tumors, which seemed to be activated by a downstream-promotion mechanism, due to an adjacently integrated provirus. As first objective for 1987, we aimed at cloning this novel mRNA in the form of cDNA, in order to determine its nature and its possible involvement in leukemogenesis.

2) In order to test for the possible participation of retroviruses in the radiation-induced leukemogenic process in C57BL/Ka mice, we looked for novel, recombinant proviruses in radiogenic lymphomas induced by a protocol in which retroviral involvement is difficult not to admit.

III. Progress achieved.

1. Methodology.

"Southern" technique to analyse tumor DNAs with molecular probes homologous to various parts of the RadLV genome.
"Northern" technique to analyse tumor polyadenylated RNA with molecular probes homologous to various parts of the RadLV genome.

2. Results.

1) Objective 1: the mechanism of leukemia induction by RadLV

We have already reported the molecular cloning of the proviral genome of RadLV/VL3(T+L+), the major leukemogenic component of the RadLV complex. The determination of the nucleotide sequence of the obtained clone allowed us to postulate a leukemogenic mechanism consisting in the activation of a cellular (onco)gene by consensus enhancer sequences present in the long terminal repeat (LTR) of the RadLV/VL3 (T+L+)genome. A major argument in favor of this mechanism arose from the finding that 10% of the thymic lymphomas, induced in rats with RadLV/VL3 (T+L+), derived from a single cell in which proviral insertion had occurred in the vicinity of the c-myc gene. However, in 90% of the virus-induced thymic lymphomas, we detected in addition to the retroviral RNAs (8.3kb and 3.4kb), a novel polyadenylated RNA of ca. 2.4kb, bearing a strong homology with only the U5 LTR probe. This novel RNA is most probably synthesized by virtue of proviral insertion adjacent to a so far unknown (onco)gene. To identify the new RNA transcript, we cloned the total cellular messenger RNA population in form of in vitro synthesized complementary DNA (cDNA library). By screening the library with the U5 probe , we could select some recombinant clones, which were U5 positive but negative for any other RadLV sequence. These recombinants were used as probes in "Nothern" hybridization to ensure they recognize the novel 2.4kb mRNA of interest. The results were negative: none of the selected clones corresponded with the novel mRNA. Sequence analysis of some of the clones revealed that the sequences that we picked up had a good homology with rat-related endogenous RNA.

2) Objective 2: appearance of novel proviruses in radiation-induced lymphomas of the C57BL/Ka mouse

A major argument in favor of a retroviral participation in radiation-induced leukemogenesis comes from the fact that C57BL/Ka mice, thymectomized at the time of leukemogenic irradiation, confer a malignant phenotype to thymus cells which are grafted after treatment. Irradiation seems to activate, in thymectomized mice, a factor (a retrovirus?) which is transferred to the graft and provokes its transformation. By molecular hybridization we could demonstrate that radiation leukemia virus-related proviral genomes appear only sporadically in primary radiolymphomas of C57BL/Ka mice, when the induction results from the irradiation of intact (nonthymectomized) animals. However, in more than 80% of the grafts that underwent

lymphomagenesis in irradiated recipients, we observed the occurence of a novel KpnI restriction fragment typical for a xenotropic provirus, but detectable with an ecotropic-specific probe. We thus demonstrated that the tumorigenic conversion of grafted thymic cells most often correlated with the acquisition of a novel recombinant provirus. This result could be an argument in favor of the hypothesis that irradiation of the host provokes the appearance of a thymotropic and leukemogenic virus, responsible for the transformation of non-irradiated grafts.
However, the proviral integration patterns were rarely clonal, rendering difficult to interprete and to investigate the mechanism by which novel proviruses might exert their leukemogenic potential, if any.

3. Discussion.

1) The mechanism of leukemia induction by RadLV.

We found that 90% of the RadLV-induced rat tumors display the presence of a novel RNA, most probably synthesized by virtue of proviral insertion adjacent to a gene which is apparently unrelated to known oncogenes. Alternatively, the appearance of this mRNA could be explained by trans activation of endogenous retroviral or retroviral-like promotors by replicating retroviruses, although such a mechanism so far has not been reported in the case of murine leukemia viruses.
If downstream promotion exists, two criteria must be fulfilled: first, RNA synthesized from cellular sequences adjacent to the proviral insertion must contain at its 5' end proviral sequences homologous to the U5 region of the LTR. Second, homology with other proviral sequences is not expected. Twelve of the fifteen RadLV/VL3 (T+L+)-induced thymic lymphomas were shown to possess a novel 2.4kb RNA which responds partially to these two criteria. The only divergence is a weak homology with a small part of the env gene of RadLV/VL3 (T+L+). So we can deduce that probably a frequent downstream promotion occurs, but we have to take into account and to explain this homology with viral sequences. At this stage, only a determination of the nucleotide sequence will help us in interpreting this result.
Using U5 LTR as a tracer, this polyadenylated RNA can be cloned to determine its nature. Our first trial to isolate the mRNA in form of in vitro synthesized cDNA lead to recombinants, which were not specific for the RNA of interest. Sequence analysis showed that the selected clones contain rat-related endogenous virus sequences. Our failure to pick up recombinant clones, which contain the sequence of interest, is probably caused by a strong homology between the U5 probe and the rat-related endogenous sequences synthesized in the tumors. It is clear that elimination of the bulk of the viral RNAs is necessary before cloning. To avoid the viral sequences we will make a new cDNA library, but this time starting from the electrophoretically purified 2.4kb polyA+ RNA. By minimizing contamination of viral RNAs by this purification step, we hopefully will succeed in determining the nature of this novel mRNA.

2) Appearance of novel proviruses in radiation-induced lymphomas of the C57BL/Ka mouse.

Radiation leukemia virus-related proviral genomes appear only sporadically in primary C57BL/Ka radiation-induced thymic lymphomas. In contrast, we actually detected the appearance of recombinant proviruses in most of the tumors originating in nonirradiated thymuses that were grafted in irradiated thymectomized hosts. The integration patterns were rarely clonal and, even when it was the case, did not show evidence for a specific integration site. So if retroviruses are implicated in the tumorigenic process it seems to be by a mechanism that was not described until now: trans activation of an oncogene or intervention of a viral product as an oncogene. Another possibility is that the grafted cells were infected after transformation. In this case, the virus would be only a passenger, which had nothing to do with the leukemogenic process. Nevertheless, it is not unreasonable to suppose that common cellular genes might be involved in radiation and retrovirus-induced leukemogenesis. So it is of the first importance that the nature of the novel mRNA detected in RadLV-induced tumors be determined. Checking wether the gene(s) from which it originate participate(s) or not to radiation leukemogenesis will be the next step in our investigation.

IV. Objectives for the next reporting period:

The novel cellular RNA , associated with RadLV-induced leukemogenesis will be molecularly cloned after elimination of the bulk of viral RNAs. The molecular clone thus obtained will allow characterization and analysis of the expression of the corresponding gene in radiogenic lymphomas.

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

Abteilung für Pathologie, Gesellschaft für Strahlen- und Umweltforschung, München (GSF), D-8042 Neuherberg, F.R.G. (Dr Günther Straus)
Department of Molecular Biology, Univ. of Aarhus, Mollers Allé 130, DK-8000 Aarhus, Denmark (Dr. F.S. Pedersen)

VI. Publications:

J. Merregaert, M. Janowski & E.P. Reddy : Nucleotide sequence of a radiation leukemia virus genome. Virology, 158, 88-102, 1987.

M. Janowski & J.M. Nuyten : Une minorité des lymphomes thymiques induits chez le rat un virus des radioleucoses murines présente une insertion à proximité de c-myc, et une majorité un nouvel ARN polyadénylé non viral. C.R.Soc.Biol., 181, 55-61, 1987.

M. Janowski : A novel nonviral RNA species in thymic lymphomas induced by the radiation leukemia virus. Arch. Internat. Physiol. Biochim., 95, B-82, 1987.

M. Janowski, B. Borremans, R. Hooghe, J. Merregaert, P. Reddy, J. Boniver & M.P. Defresne : Genes involved in murine thymic lymphomagenesis. In : Radiation Research (Proc. 8th Internat. Congress Radation Research, Edingburgh, July 1987), Taylor & Francis 1987, pp.482-487.

Title of the project no : 2

Molecular biology of radiation-induced osteosarcomagenesis.

Head(s) of project:

M. Janowski

Scientific staff:

B. Borremans
M. Janowski

I. Objectives of the project:

By analysing the topography of the newly integrated proviruses, which often appear in the murine osteosarcomas induced with bone-seeking radionucleotides, we propose to identify and characterize the genes submitted to critical alterations in relation with radiation-induced osteosarcomas.

II. Objectives for the reporting period:

In order to identify the oncogene(s) activated in radiation-induced bone tumors, we searched for tumor specific changes in the topografy of ecotropic murine leukemia proviruses in ^{90}Sr-induced bone tumors of CF1-mice and in the osteosarcoma cell line O-127a1, derived from a ^{90}Sr-induced bone tumor of CF1-mouse.

III. Progress achieved:

1. Methodology

Restriction enzyme analysis of DNA was done according to the Southern technique, using the viral ecotropic-specific Npl5E probe. Molecular cloning will be done in adequate vectors.

2. Results

The development of ^{90}Sr- and ^{224}Ra-induced osteosarcomas in mice is associated with the activation of endogenous retroviruses in bone tissues during the early latency period, which later are found to be expressed in the osteosarcomas. To check if new proviruses are integrated in tumor DNAs, which is to be expected from retroviruses involved in neoplastic transformation, we analysed tumor DNAs using the Southern blotting technique and ecotropic-specific probes. Using the Npl5E ecotropic-specific probe, we demonstrated that new integrations of ecotropic and/or recombinant ecotropic proviruses have occured in these tumor DNAs as well as in the osteosarcoma cell line O-127al. However, it was difficult to conclude for clonality of the proviral integration because of weak hybridization responses with the Npl5E probe.

3. Discussion

Integration of different ecotropic and/or recombinant ecotropic proviruses seems to occur in radiation-induced osteosarcomas of CF1-mice. This newly integrated proviruses might be responsible for the activation of neighbouring cellular sequences. If the latter are potentially oncogenic, their resulting enhanced expression could lead to neoplastic transformation. This mechanism can be tested: cloning of newly integrated proviruses together with their flanking sequences, and using thereafter the flanking sequences as molecular probes could reveal the existence of common integration domains in the investigated osteosarcomas and the osteosarcoma cell line O-127al. An actual relationship between provirus integration and malignant transformation should however be further investigated in the case of radiogenic bone tumors.

IV. Objectives for the next reporting period:

We will first try to confirm the hybridization results obtained by using other ecotropic specific probes rather than the Np15E probe (for ex.:MCF (Mink-Cell-Focus-Forming)-specific probes).
Novel integrated proviruses and their flanking sequences of the O-127al cell line will be cloned in an appropiate vector. The flanking sequences will be used as probes for screening other radiation induced osteosarcomas in order to detect common integration domains. If they exist, they should lead to the detection of gene(s) which very probably play a role in radiation-induced osteosarcomagenesis.

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

Inserm Unit 117, Fondation Bergonié, Cours de l'Argonne 229, F-33076, Bordeaux, France (Dr. B. Guillemain)
Institut für Pathologie, Gesellschaft für Strahlen- und Umweltforschung, Ingolstädter Landstrasse, 1, D-8042 Neuherberg, FRG (Dr. V. Erfle)
Department of Molecular Biology, University of Aarhus, Mollers Allé 130, DK-8000 Aarhus, Denmark (Dr. F.S. Pedersen)

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-093-IRL

Federated Dublin
Voluntary Hospitals
P.O. Box 795
IRL - Dublin 8

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 of the dosimetry of radioiodine in cell culture.

Head(s) of project: J.F. Malone.

Scientific staff: J. F. Malone
K.P. Maher
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 microdosimetric monitoring of the structure of the thyroid cell cultures using a sophisticated computer model and an image analysis computer, as well as microscopic and macroscopic experimental investigations to confirm these results.

II. Objectives for the reporting period:

(a) Extension of survival, recovery and functional studies with particular emphasis on the comparison of recovery data, dose rate effect and ^{131}I studies.

(b) Development of the cell kinetics/radiation response model.

(c) Simplification of the automated methods for quantitative histology in dosimetry.

(d) Microdosimetry studies of ^{131}I and other radionuclides of iodine.

III. Progress achieved:

The most substantial progress achieved during 1987 was with clonogenic cell survival studies particularly in comparing ^{60}Co and ^{131}I in cells of ovine and human origin. Additionally methodological developments have allowed the assay of outgrowth from explants be assayed for growth inhibition and this provides an alternative approach to growth inhibition/cell survival studies.

METHODOLOGY: The tissue culture methodology is the same as that used previously but has been extended to use an explant technique where human tissue samples are too small for conventional processing. Immunocytochemical techniques have been acquired and these are being used to assess the relative frequency of fibroblasts and epithelial cells in the cultures, as well as to define the differentiated cell population. Autoradiographic techniques have also been developed and are being compared with proliferating cell antigen cytochemical methods to assess their usefulness in de[illegible] a stem cell population should one exist.

RESULTS:

1. Several experiments comparing the effects of Cobalt 60 and ^{131}I irradiation have now been completed both for human and ovine glands. The results obtained for ovine tissue are remarkably constant and show Do values of ~2.0 Gy for Cobalt-60 and 10 Gy for ^{131}I. With human glands a marked variation has been found. Do values for Cobalt-60 range from 0.5 - 2 Gy and for ^{131}I they range from 6 to 9 Gy. The ratio of Cobalt-60 : ^{131}I responses has been determined for each human sample and is the order of 6, compared with 5 for sheep.

2. The explant technique gives rise to growth inhibition curves for thyroids which are highly resistant and have Do values similar to those previously found by Malone et al. using the weight assay. The difference between these results and the results of the clonogenic assay suggests that only a small fraction of cells which grow out from the explant are involved in significant proliferation.

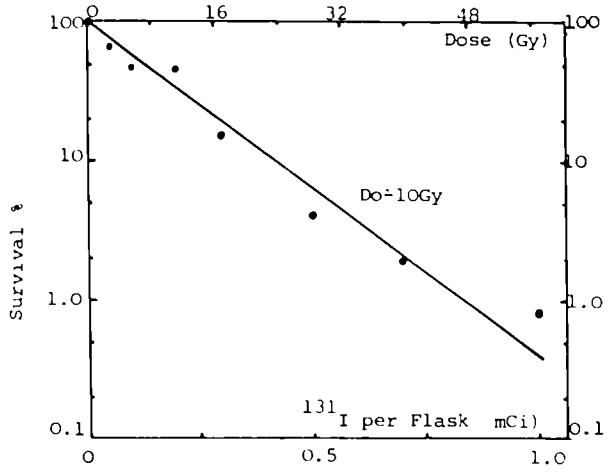


FIGURE 1:

Average of Cell Survival for 5 Sheep Thyroids after ^{131}I irradiation for 8 days.

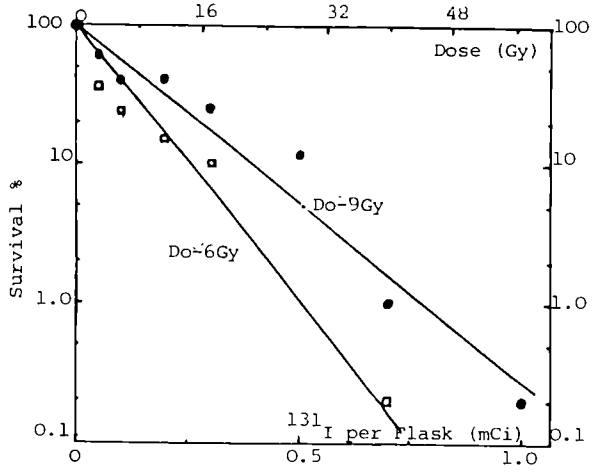


FIGURE 2:

Cell Survival for two different Human Thyroid Cultures after 8 days of ^{131}I irradiation.

This supports the model of thyroid radiation response being developed in terms of cell kinetics, and should contribute to the rational understanding of this field within a framework that is well established for other tissues. Further work has been completed on this formulation borrowing from the H-F Hybrid Tissue Model of Wheldon and Michalowski.

3. The immunocytochemical and autoradiographic techniques are being applied to these populations at present in an attempt to determine the relative frequency of proliferating units, differentiated units and stromal elements in monolayer and explant cultures prior to and after irradiation in situ and after cloning out.

4. An experimental design has been worked out for testing the degree to which the higher Do obtained following iodine irradiation is due to dose rate effects. This involves irradiating samples with Cobalt 60 at intervals ranging from every 12 hrs - 7 days and comparing the surviving fractions obtained with those found for ^{131}I. Feasibility studies to determine appropriate cell numbers to plate for these experiments have been completed. In all of these experiments a non thyroid cell line (which is more robust in its response to experimental manipulation) is being used as a control.

IV. Objectives for the next reporting period:

(1) To continue the survival and recovery studies using Cobalt 60 x-rays and ^{131}Iodine.

(2) To continue to develop the cell kinetic/radiation response model using newly acquired autoradiographic and immunocytochemical techniques. Particular emphasis will be placed on identifying a stem cell population should one exist in the culture system, and relating components of the cell population to macroscopic responses.

(3) To develop a 3-D culture model for thyroid as a means of approaching the dosimetric problem of follicular cell irradiation.

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

Dr. C. Mothersill/Dr. C. Seymour, 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
M. Lewis
B. Tuohy

I. Objectives of the project:

To determine and quantify the carcinogenic effect of acute and radio-iodine 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 the work on quantification of dose response and related matters outlined above.
2. To initiate ^{131}I and low dose rate studies in both sheep and human material using the various endpoints already described.
3. To continue the attempt to obtain human thyroid material which has been subject to <u>in vivo</u> irradiation.
4. To continue and further develop epidemiological studies.

III. Progress achieved:

METHODOLOGY: The methodology developed for the assay of transformation frequency in thyroid cultures in previous years is being applied and new endpoints which may be more useful for epithelial cell transformation studies are being sought. Soft agar growth is proving a reliable and reproducible endpoint but all attempts to form tumours in nude mice by inoculating them with agar positive cells are proving negative. The validity of this as a definitive endpoint, which holds for fibroblast systems is, however, open to question in epithelial systems since epithelial tumour cells tend to terminally differentiate in nude mice. New methods being developed include oncogene product production using immunocytochemical techniques and increased proliferation using the proliferating cell antigen Kl67. Work is continuing using LDH isoenzyme profile anlaysis.

Because of the difficulty in obtaining sufficient quantities of human thyroid tissue, this is being cultured using an explant technique.

The facility for using ^{131}I has made it possible to look for changes associated with transformation in ^{131}I irradiated cultures and these experiments are under way.

Our Group participated in the development of the Post Chernobyl initiative with special reference to the effects of ^{131}I in the thyroid.

RESULTS:

1. A total of 10 sheep thyroid cultures have been screened for soft agar positive colonies following Cobalt 60 irradiation. The results, which are quite consistent, are shown in Table 1, presented as colonies/cell exposed. Also shown in Table 1 is the correlated data to show results per cell at risk. The latter calculation takes into account the cell loss due to death from radiation. A lethal mutation term is included in the correction because of the number of cell generations which have elapsed between initial exposure of the cells and measurement of the transformation endpoint. To avoid problems resulting from proliferation of transformed cells the endpoint is scored at the first passage where it can be detected even though this may vary depending on the radiation dose.

TABLE 1

SOFT AGAR POSITIVE CLONES DETECTED IN CULTURES OF SHEEP THYROID (n=10) EXPOSED TO DOSES OF RADIATION IN THE RANGE 0 - 12.5 Gy.

DOSE	CLONES/CELL EXPOSED	PASSAGES ELAPSING TO SOFT AGAR DETECTION	SOFT AGAR CLONES/ SURVIVING* CELL
0	1.1×10^{-5} (Bgr.) for unirradiated cells.	-	(1.1×10^{-5})
2.5	9.9×10^{-5}	12 ± 2	4.1×10^{-4}
5.0	4×10^{-4}	11 ± 1	4.0×10^{-3}
7.5	2.7×10^{-4}	7 ± 2	3.9×10^{-2}
10.0	4.0×10^{-5}	5 ± 1	5.1×10^{-1}
12.5	2.5×10^{-5}	4 ± 1	8.5×10^{-1}

*Survival here is residual survival calculated by taking the plating efficiency drop at each passage into account as in Mothersill and Seymour (1987), Int. J. Radiat. Biol., 4 723-729.

2. Transformation using the soft agar endpoint has still not been detected in human cultures although, as reported last year, several other transformation associated changes have been found. Work with human cells is now being concentrated on the detection of oncogene products and isoenzyme shifts using the concepts and techniques being developed in projects being run by the Saint Luke's Hospital Group (B16 092 and 184).

3. Seven trials including 5 sheep thyroid cultures and 2 human thyroid cultures have been concluded in an attempt to detect any changes associated with transformation. In all cases senescence of the irradiated cells occured before senescence of the control cells and no cells survived long enough for transformation to be looked for. The senescence phenomenon was not the same as loss of clonogenic ability and involved morphological and reproductive changes generally associated with normal epithelial cells which have undergone their allowed number of cell divisions. It could most usefully be termed 'premature senescence'. It is not clear at present what mechanism underlies this but attempts to check for dose rate effects and lethal mutation frequencies are under way using the experimental design developed in Project 1.

4. A complex longterm experiment to seek transformation after ^{131}I irradiation has been designed. As a pilot run to ensure the success of the experiment, and provide a non-thyroid cell control, it is being performed in the first instance with CHO cells. This is technically less difficult and not as open to misinterpretation of results.

IV. Objectives for the next reporting period:

1. To continue to quantify the dose response relationship.
2. Using the new ^{131}I facility to attempt to confirm the apparent absence of transformation following ^{131}I irradiation, and to investigate the mechanism underlying this observation.
3. To apply immunocytochemical methods to the study of early carcinogenic change in the limited amounts of human thyroid tissue available.
4. To continue the attempt to obtain human thyroid material which has been subject to in vivo irradiation.
5. To continue and further develop epidemiological studies.

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

Dr. C. Mothersill, Dr. C.B. Seymour, St. Luke's Hospital, Dublin 6.

Prof. J.E. Dumont, Hospital Erasme, Brussels.

Prof. J.S. Orr, London.

Prof. M.J. Cullen, St. James's Hospital, Dublin 8.

VI. Publications: IN SCIENTIFIC JOURNALS/MONOGRAPHS:

Longterm effects of gamma irradiation on cultured human thyroid cells.
C.B. Seymour and C. Mothersill.
Int. J. Radiat. Biol. (1987), 51 (3), 381-91.

Radiation induced outgrowth inhibition in explant cultures from surgical specimens of five human organs.
C. Mothersill, A. Cusack and C.B. Seymour.
British Journal of Radiology (1987) (in press)

Lethal mutations attributable to misrepair of Q-lesions.
Alper, Tikvah, Mothersill, Carmel; and Seymour, C.B.
International Journal of Radiation Biology, (1987), (in press)

OTHER PRESENTATIONS INCLUDED:

Dosemetric and Radiobiological Aspects of Thyroid Response to ^{131}I at the cellular level. (1987) Special Symposium of Thyroid Radiation Response at the European Thyroid Association, Lausanne, Switzerland (to be published).
J.F. Malone.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Dr. R. Masse
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.: B 16-096-F

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:

Déterminer la valeur des coefficients de risques relatifs et absolus après irradiation chez le rat, et étude des effets combinés.

II. Objectives for the reporting period:

1) Objectif expérimental:
Etudier sur le poumon l'action d'un agent promoteur spécifique après irradiation en regardant les effets combinés de différentes doses d'initiateur et de promoteur sur l'apparition de cancers pulmonaires chez le rat Sprague-Dawley.
2) Objectif d'analyse des données:
Faire une étude statistique des données expérimentales obtenues depuis 1985, afin de savoir si le risque encouru est de type relatif ou absolu.

III Progress achieved:

IMPORTANCE RELATIVE DES FACTEURS D'INITIATION ET DE PROMOTION

1) Méthode

3 groupes de rats sont irradiés:

-le premier groupe avec des neutrons de fission dans une gamme de doses de 15 à 220 cGy.

-le deuxième groupe avec des gamma du Cobalt 60 dans une gamme de doses de 400 à 1600 cGy.

-le troisième groupe inhale de 50 à 2240 WLM de radon.

Ces trois groupes reçoivent, 1 mois après la fin de l'irradiation globale ou de l'inhalation, des injections intramusculaires de 5-6 Benzoflavone (promoteur spécifique des cancers pulmonaires épidermoides) à raison de 1 injection de 25 mg/kg tous les 15 jours à différentes doses. Les rats sont tués 1 mois après la fin des injections de BNF.

2) Résultats

-résultats expérimentaux (tableau)

-INCIDENCE DES CANCERS PULMONAIRES CHEZ LE RAT SPRAGUE-DAWLEY APRES ACTION COMBINEE D'UNE INITIATION PAR IRRADIATION ET D'UNE PROMOTION SPECIFIQUE PAR LA BENZOFLAVONE (BNF).

		NOMBRE D'INJECTIONS 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	
(cGy)	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	

Une dose minimale d initiateur et de promoteur est nécessaire pour que les cancers apparaissent. Les doses d initiateur et de promoteur nécessaires évoluent en sens inverses.

ANALYSE STATISTIQUE DES DONNEES

1) Méthode

L'analyse statistique des données est réalisée suivant la méthode mise au point 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.

2) Résultats

Chez le rat, comme chez l'homme, l'excès de cancers par tranches d'âges 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. On peut donc affirmer que le risque n'est pas de type absolu. Le mode de risque relatif, dans lequel existe un facteur de multiplication constant pour toutes les doses, 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; les deux grands types de tumeurs se séparent bien.

Les sarcomes et les carcinomes ont ensuite été séparés en deux groupes. L'un comportant les organes profonds et l'autre les tissus mous. Dans les deux cas, les pentes sont comparables.

Etant donné le trop petit nombre de données pour chaque organe, il n'a pas été possible d'analyser statistiquement les différents organes séparément. Cette analyse a cependant pu être réalisée pour les carcinomes pulmonaires, et les irradiations par le radon ont pu être comparées à celles produites par des gamma et des neutrons.

Les carcinomes pulmonaires ont un comportement comparable à celui de l'ensemble des autres carcinomes.

3) Discussion

Les données expérimentales sont en accord avec la méthode d'analyse utilisée dans le récent rapport BEIR IV pour traiter les données biologiques épidémiologiques sur le radon.

IV Objectives for the next reporting period:

Analyse statistique des résultats.

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

VI Publications

Rapport DPS/IPSN 1986 pp.185-186,191-192
1987 en cours de publication.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

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

Contract no.: BI6-D-100-UK

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:

Synergistic effects of cigarette smoke in the induction of lung tumours by inhaled actinides.

List of projects:

1. Synergistic effects of cigarette smoke in the induction of lung tumours by inhaled actinides.

Title of the project no.:

BI6-D-100-UK

Synergistic effects of cigarette smoke in the induction of lung tumours by inhaled actinides.

Head(s) of project:

A. Morgan

Scientific staff:

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 $^{239}PuO_2$. The original proposal was for a five year study. However, funding was agreed for a two year period with possible subsequent support being conditional on the satisfactory completion of a two year pilot study. This final report describes the results of the two year study.

II. Objectives for the reporting period:

To compare 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.

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 m 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}Pu. At 8 days after inhalation the animals were whole body counted for ^{241}Am - a minor contaminant of the ^{239}Pu - to determine individual IADs. On the basis of these counts the animals were allocated to matched cage groups and each cage was 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, UK, 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 - that is at 18 months after their exposure to plutonium - the mice in all three groups were sacrificed. Their lungs were then inflated, via the trachea, with 0.8 ml of fixative, removed from the thorax, dehydrated in ethanol and cleared using methyl salicyclate. Subsequently, they were examined at low magnification by transmitted light microscopy and any lung lobes containing opacities (mostly tumours) were excised and embedded in paraffin wax for histopathological examination. Lung opacities as small as 0.5 mm could be detected by this technique. The pathological examination and classification of lung opacities is currently being carried out under contract, according to GLP standards, by Paul N. Brooks (Consultant in Experimental Toxicology and Histopathology). In addition to the above, the normal lungs from each experimental group were analysed to determine their radionuclide content at death.

2. RESULTS

During the course of the experiment the caged-control mice continued to gain weight. In contrast, those animals that were either exposed to cigarette smoke or sham exposed to smoke gained little weight during the 12 month smoke-exposure period (3 g), but grew rapidly during the post-exposure period prior to sacrifice gaining a further 6 g in six months. These values compare with the caged control animals which gained ~ 11 g during the first year of the experiment and then little or no weight during the final six month period.

Examination of the lungs of the animals 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. However, the numbers of lesions found in the different lung lobes was very variable: lobe 1 - 11, lobe 2 - 17, lobe 3 - 9, lobe 4 - 3, and lobe 5 - 22. These numbers were related to the size of each lobe: 1 - 19.4mg, 2 - 36.9mg, 3 - 22.4mg, 4 - 14.2mg, 5 - 47.6mg.

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
PCC	43	30	53.7%
PSS	45	21	51.4%
PTS	38	10	26.3%

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.

Table 2 shows the amount of ^{239}Pu remaining in the lungs of the mice in each group at the end of the experiment. The lungs used for the determinations were those of the 74 mice found to contain no macroscopically discernible lesion. It can be seen that the lung contents of the cage-control and sham-exposed mice were very similar, but that the lungs of animals exposed to cigarette smoke contained approximately four times as much plutonium, indicating that the cigarette smoke inhibited the clearance of the radionuclide. Therefore, the lungs of the animals exposed to smoke will have received a substantially larger radiation dose than those of the mice in the control groups. Also, given the disparity between the weights of the cage-control and sham-exposed mice, it would seem that stress and exercise - which are considered likely to have limited the weight gain of the sham exposed (and tobacco smoke exposed) mice - have not effected plutonium mobilisation within the lungs.

3. CONCLUSIONS

From the above it is evident that, due to the absence of of the results of the final histopathological examination of the excised lung lesions, no final conclusions can be drawn from the results of this study at the present time. However, it is considered unlikely that the histopathology reports will substantially alter the tumour incidences indicated by the lesion data described above. Consequently, some preliminary conclusions may be drawn, namely that:

1. - The frequency of lung lesions in the experimental mice is likely to be greater than was found in an earlier study where the animals were sacrificed at 12 rather than at 18 months.

2. - It is very unlikely that the results of the experiment could be used to support the hypothesis that the effects of tobacco smoke inhalation and plutonium inhalation are synergistic with regard to the production of lung tumours.

Experimental Group	Initial Alveolar Deposit	Alveolar Deposit at Sacrifice
PCC	100 Bq	3.3 ± 1.1 Bq
PSS	100 Bq	3.8 ± 0.9 Bq
PTS	100 Bq	14.2 ± 2.6 Bq

Table 2. The ^{239}Pu contents of the lungs of the experimental animals at the beginning and end of the experiment. The values for the sacrifice lung burden are presented as a mean and standard error.

The results of a previous study undertaken in order to determine the dose response relationship for lung tumours following the inhalation of a $^{239}PuO_2$ aerosol, in the same strain of mouse as used for the current studies, showed a peak incidence of tumours following the accumulation of a lung burden of 160Bq. At this level of plutonium intake a lung tumour incidence of 0.36 tumours per mouse was found in a group of 48 mice. This compared with a frequency of 0.23 tumours per mouse in a similarly sized batch of animals given an IAD of 90 Bq - ie. close to the IAD used in the synergy study - and of 0.07 tumours per mouse in the no plutonium control group of 235 mice. All of these animals were sacrificed at one year post-exposure to plutonium. In contrast, the preliminary results of the synergy study - where the animals were sacrificed after 18 months - indicate a tumour frequency (0.47 - 0.70) at an IAD of 100 Bq that is up to two times higher than might have been predicted on the basis of the dose response study (Table 1). It

would seem, therefore, that the decision to prolong the study to 18 months, rather than sacrifice the animals at one year as originally intended - in order to see a higher tumour frequency was justified. However, this extension of the project has rendered the original dose response study inappropriate as a control for the synergy study and a second project has been initiated in order to fill the gap. For this study five groups of 58 mice have been exposed to $^{239}PuO_2$ to give final IAD's of either 50, 75, 110, 170 or 350Bq. These animals will be sacrificed during 1989.

When the results of the synergy study (Table 1) are examined it is evident that the number of lesions found in the mice exposed to cigarette smoke was lower than was found in either of the other groups of animals which received plutonium only. This result may be interpreted in two ways. Firstly, it may be interpreted as indicating that the effects of alpha-particles and cigarette smoke on the lung are antagonistic and that cigarette smoke has a protective rather than synergistic effect. Such a conclusion would not be consistent with the results of similar studies conducted with rats using radon as the source of alpha-particles (Chameaud et al., 1979), but would accord with the results of dog studies conducted at Battelle Pacific Northwest Laboratories in the USA (Cross et al., 1982) where a combination of exposure to alpha-particle and cigarette smoke also produced fewer effects than the alpha-particles alone. Alternatively, it may be that the promoting effect of cigarette smoke, together with the greater radiation dose to the lungs of the mice which received tobacco smoke, due to impaired clearance of plutonium, may have had a lowering effect on the tumour incidence because of the consequent increased sterilisation of transformed cells. Such an explanation is reasonable bearing in mind that in the mice killed at one year after exposure to plutonium the peak tumour incidence corresponded to an IAD of 160 Bq which is not much greater than the 100 Bq used for the present studies. If so it is axiomatic that in order to establish the true effects of cigarette smoke on the incidence of lung tumours in plutonium exposed mice it will be necessary to repeat the synergy study using much lower doses of plutonium. Such repeat studies are considered worthwhile, due to the prevalence of cigarette smoking in radiation-exposed populations and are currently being undertaken.

4. REFERENCES

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Cross F.T., Palmer R.F., Filipy R.E., Dagle G.E. and Stuart B.O. (1982). Carcinogenic effects of radon daughters, uranium ore dust and cigarette smoke in beagle dogs. Health Phys. 42, 33.

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

None

V. Publications:

Black A., Matulionis D.H., Moores S.R., Morgan A., Talbot R.J. and Walsh M. (submitted for publication). Morphological and physiological responses of the mouse lung following chronic exposure to cigarette smoke. Exptl. Lung Res.

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RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

St. Luke's Hospital
Highfield Road
Rathgar
IRL - Dublin 6

Contract no.: BI6-D-092-IRL

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 L. Smith
Ms C. O'Neill

I. Objectives of the project:

(1) To develop methods for the 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 investigations of the response of bladder, oesophageal and breast explant outgrowths to irradiation with particular emphasis on autoradiographic and cytophotometric analysis.

(2) To continue to study the LDH isoenzyme profile of normal, tumour and irradiated oesophageal and bladder epithelium and to commence screening for altered GST activity.

(3) To continue to develop collagen gel or ECM three dimensional cultures and to develop a quantitative method of analysis of such cultures.

(4) To develop a model for transformation dose response using available data in the laboratory and in the literature, which incorporates a lethal mutation term.

(5) To continue ultrastructural analysis of normal, tumour and irradiated explant outgrowths with particular attention to longterm changes in mitochondrial and cell cell junctions.

III. Progress achieved:

METHODOLOGY: (i) The tissue culture methodology has been optimised with regard to media, sera, hormones and other additives for oesophageal epithelium and a three-fold increase in cell number obtainable has been achieved using the optimised medium compared with the conventional one. The optimisation procedure is now being applied to the other tissues being cultured.
(ii) Efforts have been made to secure regular supplies of normal healthy tissue where no disease is present. The most promising sources are urothelium associated with donor kidneys for transplantation and cervical and ovarian tissue removed during total hysterectomy operations.
(iii)New techniques which are now being applied to determine cellular radiation responses which may indicate early carcinogenic change include immunocytochemical analysis of explant outgrowths in situ for oncogene products, proliferative ability and occurrence of abnormal or unusual intermediate filaments. Ultrastructural analysis of morphological changes is also continuing. The staff benefited from two technical visits/courses aimed at gaining expertise in these techniques; Ms A. Cusack spent one month at the German Cancer Research Institute in Heidelberg, studying techniques for isolating and identifying malignant prekeratins in our explant tissue and cultured cellular outgrowths and Dr Mothersill attended a two week course in the Histopathology Dept. of Hammersmith Hospital on Modern Immunocytochemistry.
(iv) Techniques for 3-D culture are being applied to urothelium and oesophageal mucosa. To date cultures have successfully organised in collagen gels and methods of analysing the preparations are being developed.

RESULTS: (1) The radiation response outgrowth endpoint developed earlier has now been shown to give meaningful and reproducible results for ten human epithelial tissues in culture. Of these, endometrium and breast have proved to be most radiosensitive and thyroid most radioresistant. Tumour tissue cultures used as positive controls in carcinogenesis studies have always proved highly resistant to radiation relative to their normal counterparts Fractionating the dose of radiation increased the survival, indicating that the method can detect recovery where present. Immunocytochemical analysis confirmed the epithelial nature of the cultured cells and autoradiographic analysis was used to show that cell proliferation was being measured and not simply migration or increase in cell size. These techniques are also being applied to quantify the response to radiation and to identify the subpopulations of cells which show changes in proliferation rate following irradiation.

(2) Analysis of LDH isoenzyme and GST are still being done using oesophageal mucosa and urothelium but considerable difficulties are being experienced due to the low cell numbers obtainable after explants have been irradiated. Changes in the LDH profile of irradiated explants have been noted and where growth is not severely inhibited by radiation, shifts have also been noted for cells. In all cases the shift is from the normal aerobic pattern where bands 1 and 2 only are seen to a more anaerobic profile where bands 3, 4 and 5 may be detected. Efforts are being made to find a supplier of antibodies to LDH 1 and 5 subunits in an attempt to develop an in situ cytochemical assay. Work with GST using the system is also hampered by low cell numbers.

(3) Spatial organisation of epithelial cells is highly important for maintenance and control of differentiation and for this reason we have put

considerable efforts into developing 3-D culture models where this organisation is preserved. We have succeeded in getting oesophageal epithelium and urothelium to stratify using a floating collagen gel system. Various methods of quantifying the radiobiological response are now being considered. These include sectioning the gel and staining for proliferating cells, oncogene activation etc., as well as a number of methods based on volume of cells determinations.

(4) The occurrence of lethal mutations and their relevance to survival, transformation and repair of cellular damage following irradiation is still continuing. The results suggesting that artificially low estimates for transformation in the C3H 10T½ system may be obtained if a lethal mutation term is not included in the calculation have been confirmed. The lethal mutation fraction now appears to result from error prone fast repair of damage in the low dose or shoulder region of the survival curve. Investigations are concentrating now on determining the relationship between dose, induction of repairable damage and number of successful cell divisions occurring before lethal mutations are expressed. The effect of dose rate and dose fractionation on induction of lethal mutations is also under investigation and early indications are that the level of recovery following fractionation does not reflect the size of the single dose survival curve shoulder if curves are corrected for residual damage due to lethal mutations.

(5) Studies are continuing to look for long-term ultrastructural changes in normal cells following irradiation which might be associated with carcinogenesis. To date considerable changes in nuclear morphology have been detected following low dose irradiation and an increase in number of nuclear pores has also been found. The major cytoplasmic changes include mitochondrial degeneration and proliferation of tonofibrils. Cultures of tumours of the tissues under investigation have been used as positive controls. Preliminary attempts to quantify the changes using immunogold labelling and serial sections are under way.

DISCUSSION: The results suggest that the approaches needed to assess potential radiation transformation in epithelial cells are very different to those suitable for fibroblast systems. This is partly due to the limited life span of the majority of epithelial cells in tissues which makes normal culture methods and transformation assays unsuitable. The immunocytochemical techniques are a very powerful tool for investigation of oncogene product production and other biochemical changes since they can be applied to very small cell numbers and can give information on the spatial distribution and proliferative status of positive cells. If such techniques can be applied to 3-D cultures it should be possible to develop meaningful *in vitro* models for epithelial cell transformation which will permit the radiation microdosimetry to be correlated with transformation.

The lethal mutation work has considerable implications for the construction of models of cellular transformation, many of which involve the concept of misrepair or error prone repair. It is likely, given the association between high levels of repair and high levels of lethal mutations, that lethal mutations will need to be considered in future radiation transformation models.

IV Objectives for the next 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.

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

Prof. K. Tipton, Department of Biochemistry, University of Dublin, Trinity College, Dublin 2.
Prof. J.V.McLoughlin, Department of Physiology, University of Dublin, Trinity College, Dublin 2.
Dr T. Connors, MRC 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.

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Mothersill, Carmel, Seymour, C.B., Cusack, A., O'Brien, A., Moriarty, M. and Hennessy, T.P. Differential response of human normal and tumour tissues in culture to radiation in combination with cytotoxic agents. Meeting of Cell, Tissue and Organ Culture Society, Athens, September 1987.

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Mothersill, C., Seymour, C.B., Cusack, A., O'Brien, A., Moriarty, M. and Hennessy, T.P. Differential response of human tumour and normal tissues in culture to radiation in combination with cytotoxic agents. Proc. 8th International Congress of Radiation Research, Edinburgh, 1987.

Seymour, C., Mothersill, C., Cusack, A., McDonnell, M. and Moriarty, M. A model for the determination of radiation sensitivity in primary cultures of human cells. Proc. 8th International Congress of Radiation Research, Edinburgh, 1987.

O'Donnell, C., Mothersill, C., Moriarty, M. and Seymour, C.B. The influence of lethal mutations on the 'survival' of cells exposed to fractionated doses of ionising radiation. Proc. 8th International Congress of Radiation Research, Edinburgh, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-101-F

Institut de Protection et de
Sûreté Nucléaire
CEN de Fontenay-aux-Roses
B.P. n° 6
F - 92265 Fontenay-aux-Roses

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-Salpetriere Hospital PARIS.

I. Objectives of the project:

Experimental data of inhaled particle deposition and retention in human airways are needed to evaluate inhalation toxical hazards, for the workers and for the populations. In order to ascertain the dosimetric calculations and their limits, lung modelling has to include biological variability, owing to age, sex, body size and weight, lung function indices and other such parameters.

II- Objectives for the reporting period

Total deposition of inert particles in the respiratory tract has already been studied in healthy adult and children subjects. A previous work allowed to relate the percentage deposited to individual respiratory parameters. In order to precise these relationships, various ventilation conditions have been applied in details upon trained subjects, able to simulate the various physiological features.

Regional deposition study was also undertaken with the measurement of nasal deposition during nasal breathing in healthy adults and children; these subjects went also through nasal resistances tests performed at a few respiratory flow-rates.

The data to be obtained by mouth-breathing and nose-breathing at various ages, will help to ascertain the deposition calculated models by experimental values.

III- Progress achieved

1- Influence of ventilation parameters upon total particle deposition in the respiratory tract, by mouth breathing

1-1 *Materials and methods*

A group of 5 healthy non smoker adults, 2 men and 3 women were trained to control a wide range of breathing patterns. Particle deposition of polystyrene beads (1,1.9 and 2.55 μm MMAD) was measured by comparison of inhaled and exhaled air concentrations ($D = 1 - \frac{Ce}{Ci}$). Respiratory function, lung volumes,

flow-rates and airway resistances were measured for each subject, and the ventilation during inhalation was measured by a Fleisch pneumotachograph. Breathing patterns included three tidal volume values (TV= 0.5, 1, 1.5 l), each one breathed at 4 different frequencies (f = 5, 7.5, 15, 30 x min^{-1}) with inspiratory times Ti of 6,4,2,1 s. Each subject performed twice the same test and provided a total of 72 deposition data.

Data were studied graphically for each subject and the whole group set of values was treated by the non-parametric Friedman rank sums test of an eventual increasing influence of TV and Ti upon particle deposition.

1-2 *Results*

The means values of the actual TV and Ti obtained for each breathing asked to the subject are given in Table 1. Evolution of particle deposition with the change of ventilation is shown graphically for one subject in Figure n° 1. Obviously the relationship of VT/deposition is better than the one of Ti/deposition; the relationship reliability is described by the correlation coefficient, r; it is better for high frequencies (Ti= 1 and 2 s). After 2 s, increasing Ti does not increase deposition substantially. The statistical analysis of the influence of TV upon the 3 particle size deposition (table 2) confirms the graphic observations for the 5 subjects; when the Ti influence is studied, the tests are negative.

Table 1 : RESPIRATORY PARAMETERS, TIDAL VOLUME, TV (l), AND INSPIRATORY TIME Ti (s) : OBTAINED MEAN VALUES FOR 5 SUBJECTS, IN EACH EXPECTED CLASS

Classes TV / Ti		1 s		2 s	
0.5 l	$\bar{x}$	0.50	0.83	0.50	2.17
	σ	0.034	0.12	0.055	0.14
1 l	$\bar{x}$	0.89	0.82	0.96	2
	σ	0.076	0.07	0.061	0.19
1.5 l	$\bar{x}$	1.36	0.60	1.14	1.99
	σ	0.062	0.08	0.068	0.21
Classes TV / Ti		4 s		6 s	
0.5 l	$\bar{x}$	0.17	4.13	0.50	6.64
	σ	0.017	0.29	0.067	0.72
1 l	$\bar{x}$	0.95	4.12	0.95	6.31
	σ	0.019	0.21	0.049	0.31
1.5 l	$\bar{x}$	1.17	4.15	1.37	6.06
	σ	0.050	0.42	0.081	0.73

Fig. 1 : Individual values for total deposition versus ventilation

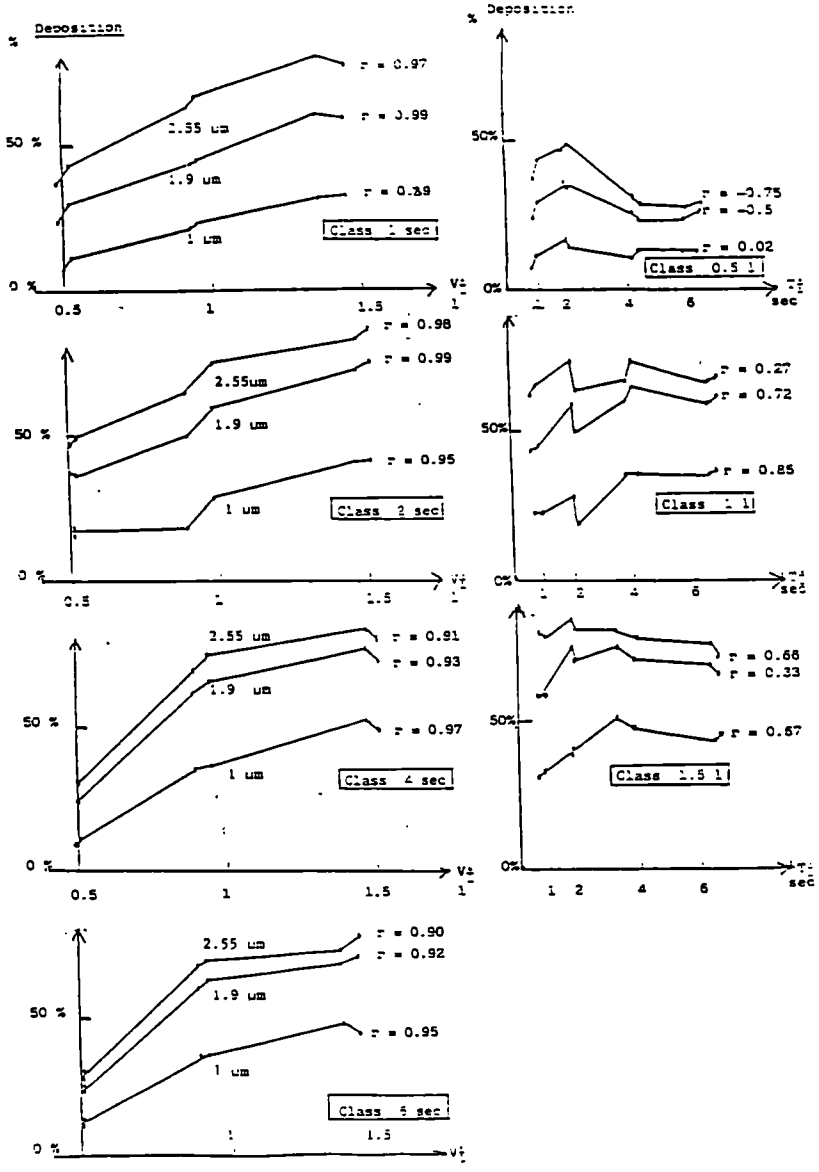

Table 2 : INFLUENCE OF TIDAL VOLUME (TV) INCREASE UPON TOTAL DEPOSITION - FRIEDMAN RANK SUMS TESTS

Test n° 1 :

Classes Ti sec / TV l	1 0.5 ; 1 ; 1.5	2 0.5 ; 1 ; 1.5	4 0.5 ; 1 ; 1.5	6 0.5 ; 1 ; 1.5
MMAD um 4.1	not equivalent $p^{=} = 0.008$	equivalent	equivalent	equivalent
1.3	not equivalent $p^{=} = 0.001$	not equivalent $p^{=} = 0.008$	equivalent	equivalent
2.55	not equivalent $p^{=} = 0.001$	not equivalent $p^{=} = 0.008$	equivalent	equivalent

$p^{=}$ = probability for equivalence

Test n° 2

Classes Ti sec / TV l	1 0.5 → 1 → 1.5	2 0.5 → 1 → 1.5	4 0.5 → 1 → 1.5	6 0.5 → 1 → 1.5
MMAD um 4.1	increasing effect $0.001 < p^{=\!=} < 0.01$			
1.3	increasing effect $p^{=\!=} = 0.001$	increasing effect $0.001 < p^{=\!=} < 0.01$		
2.55	increasing effect $p^{=\!=} = 0.001$	$0.001 < p^{=\!=} < 0.01$		

$p^{=\!=}$ = probability for not increasing effect

1-3 *Discussion*

For this particle sizes, deposition occurs both by impaction and sedimentation. In our study, where breathing flow-rate is never constant, impaction is predominant and almost instantaneous; it seems to be achieved within 2 seconds; during that time, increasing the tidal volume also increases airway surfaces, and the probability of particle deposition.

2- Regional deposition study during nose-breathing

2-1 *Materials and methods*

With the same aerosols and controlled ventilation technics a nose-breathing method was developed. Two deposition measurements are made in sequence : the first one while the subject inhales and exhales through mouth with a nose-clip, the second one while he performs the same test, but through the nose, with a nose-piece, with the mouth kept shut. After the two deposition data are obtained, the efficiency of the nose as a filter can be expressed as follows :

$$En = 1 - Cne = (1 - x) (1 - x') (1 - y) \qquad [1]$$

with :

Cne = particle concentration in expired gas through nose
x = % of particles deposited during inspiration through nose
x' = % of particles deposited during expiration through nose
y = % of particles deposited in the lower respiratory tract.

Assuming that the percentage of particles deposited in the nose is approximately the same during inspiration and expiration, and that the percentage deposited in the lower respiratory tract is the same as the one deposited when the aerosol is inhaled through the mouth (the mouth having a very low resistance) the percentage of particles deposited in the nose during inspiration can be calculated by [1].

A group of 2 adults and 5 children, male and female of various ages, underwent twice the test. Their total nasal resistances (right plus left) were measured during spontaneous quiet breathing : two nasal pipes, were connected to 2 membrane-type pressure transducers, one measuring the pressure drop in one nostril, the other, linked to a Fleish-tube measuring nasal airflows through the other nostril. The data are given in terms of air-resistance as a function of airflow.

Particle retention was measured during controlled breathing at various TV, f and flow-rates.

2-2 *Results*

Individual nasal resistances increase with respiratory flow-rate, and the children values are higher than the adults' (table 3). Inspiratory nasal deposition values are displayed in table 4 : the 2 adults have increased deposition for increasing particle size and with increasing flow-rates, and nasal resistances. For the 5 children, data are dispersed, especially for the youngest who seemed to have difficulties to manage with the nose-breathing pattern.

Table 3: INDIVIDUAL NASAL RESISTANCES VERSUS INSPIRATORY FLOW-RATE

1°/ Adults

Subject n°		Sex	Nasal resistances in kPa.l^{-1}.s at a flow-rate of : 150 ml.s^{-1}	250 ml.s^{-1}	500 ml.s^{-1}
1		M	0.191	0.248	0.388
2		F	0.125	0.156	0.234

2°/ Children

Subject n°	Age Y	Sex	Nasal resistances in kPa.l^{-1}.s at a flow-rate of : 150 ml.s^{-1}	250 ml.s^{-1}	500 ml.s^{-1}
3	7	M	0.431	0.609	
4	9	M	0.294	0.417	
5	12	F	0.308	0.407	
6	13	F	0.316	0.419	
7	14	F	0.190	0.260	

Table 4 : INDIVIDUAL NASAL EFFICIENCY IN PERCENT OF INHALED PARTICLES AS A FUNCTION OF RESPIRATORY PARAMETERS

1°/ Adults

TV ml ; f.min^{-1} ; flow-rate ml.min^{-1}	500 15 250			500 25 417			500 30 500			1000 15 500			1000 30 1000		
Particle MMAD µm	1	1.9	2.55	1	1.9	2.55	1	1.9	2.55	1	1.9	2.55	1	1.9	2.55
Subject n° 1	0.2	15.6	21.3	5.6	13.1	24.2	4.0	22.8	28.8	15.5	32.5	36.5	20	39.5	46.2
2	7.4					11.9	2.5	15.3	17.7	13.0	21.7	22.6	15.6	24.3	31.2

2°/ Children

TV ml ; f.min^{-1} ; flow-rate ml.min^{-1}	500 15 250			500 25 417			350 20 233			350 30 350			250 25 208		
Subject n° 3													6.8	13.1	13
4							3.4	9.1		10.6	14	5.4			
5							7.9	13.7	12.1	6.7	12.1	14.2			
6	14.3	23.5	26.3	20.4	26.6	28									
7	20.3	23.4	24.9	13.5	22.7	26.2									

2-3 *Discussion*

The small number of data does not allow to study any relationship between nose deposition and resistances. The resistance values are varying also probably with the nasal cycle (right and left physiological air-flow alternance) and with many environmental factors from one moment to another during the same day. They are suspected of influencing predominantly deposition in the nasal passages, and ought to be measured as closely as possible from the deposition.

Deposition data should be separated between age groups in children and compared to the adult's as was done previously for the total deposition study.

IV- Objectives for the next reporting period

In the inert particle inhalation studies, it is now technically possible to separate the nasal fraction from the total deposition in the human airways. Acquisition of such data among adult and children subjects will provide a normal range of values for these regions, at rest and at various level of ventilation, to ascertain the deposition models of particles. It is also important to try to relate them to respiratory function, particularely to airway resistances.

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.

2. Institut für Biologie
   Abteilung für Biophysikalische
   (Dr W. STAHLHOFEN)
   Strahlenforschung
   Paul-Ehrlich-Strasse 15 u. 20
   D-6000 FRANKFURT/MAIN

3. Dipartimento di Fisica, Professor V. PRODI,
   Universita Degli Studi di Bologna,
   40126 BOLOGNA.

VI. Publications:

M.H. Becquemin, M. Roy, D. Robeau, S. Bonnefous, J. Piechowski, A. Teillac. Inhaled particle deposition and clearance from the normal respiratory tract. Respir. Physiol. 1987. 67, 147-158.

M.H. Becquemin, M. Roy, S. Herson, P. Godeau, A. Teillac. Clairance tracheobronchique à court-terme dans le syndrôme de Gougerot-SjÖgren apparemment pur. Bull. Eur. Physiopathol. respir. 1986, 22, 551-557.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-201-F

Université Paul Sabatier
Faculté de Médecine Purpan
Allées Jules Guesde, 37
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Head(s) of research team(s) [name(s) and address(es)]:

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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.: Subproject n° 1a

Study of radiation hormesis in human embryonic lung fibroblasts at very low doses of radiation.

Head(s) of project:

Dr. F. CROUTE

Scientific staff:

Dr. Y. GAUBIN

Miss B. PIANEZZI

I. Objectives of the project:

Contrary to deleterious effects of high doses of ionizing radiations, very low dose rates of chronic irradiations can have a stimulatory effect on proliferation of human fibroblasts cultivated in vitro (Croute 1986). Stimulating effect could be due to the activation of radio-induced radical scavengers (catalase), giving rise in a second time to an increase of glucose consumption, either by the way of the pentose phosphate shunt or by glycolysis. On the other hand, investigations will be performed on the radiation-induced repair effects by comparison between normal diploid and mutant DNA repair deficient fibroblasts.

II. Objectives for the reporting period:

Investigations were carried out to understand the two competitive actions of very low dose rate of chronic irradiation : inhibitory and stimulatory effects.
In order to appreciate the toxic effect of the radiolysis products of the extracellular medium, the effect of irradiated media on cell proliferation was investigated.
To understand the stimulatory effect, we have studied the energetic metabolism linked to the glucose consumption i.e. the glucose uptake and the enzyme activities of glucose-6-phosphate dehydrogenase (a key enzyme of the pentose phosphate shunt) and of pyruvate kinase (a key enzyme of glycolysis).

III. Progress achieved:

I. METHODOLOGY

Human fibroblasts derived from embryonic lung were used. The cells were routinely grown in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, glucose 5g/l and 20 μg/ml gentamycine, and incubated at 37°C in humidified air containing 5% CO_2

The radioactive source was Thorium nitrate crystals set in thin and uniform layer under the cell culture flasks. The dose rate was above 200 mGy/year. As 2 mGy/year is the average natural irradiation dose absorbed by control cultures, the dose rate used was 100 times higher.

II. RESULTS AND DISCUSSION

A.- Culture conditions in which growth inhibitory effect appears.

Table I shows that the chronic irradiation of culture fibroblasts plated at a low density (5.10^5 cells/flask) causes a decrease in cell proliferation that does not appear in cell cultures plated at a higher cell density (1.10^6 cells/flask).
Fig. 1 exhibits the specific role of the irradiated extracellular medium. Cells that have been exposed to pre-irradiated medium (absorbed dose : 5 mGy) show a growth inhibition when the plating density was low ($15 . 10^4$ cells/dish). This inhibitory effect disappears for the highest cell concentration (22.10^4 cells/dish). There is no effect for absorbed doses below 3 mGy.
We can assume that inhibitory effects of these low levels of chronic irradiation can be related to the radiolysis products of the aqueous culture medium. Their toxic effects appear only with low cell densities i.e. when cell cultures exhibit a low level of scavengers (enzymes activities as catalase, peroxidase and superoxidismutase).

B.- Why a stimulatory growth effect ?

- To search the possible release of a growth factor in the medium under irradiation, we have analyzed the relative mitogenic activities of supernatants of control or irradiated quiescent cell cultures (Fig. 2). We observe that cell proliferation decreases when cells are growing in supernatant of irradiated cell cultures. We believe that this result can be related to the higher consumption of metabolic precursors in irradiated cultures due to the probable higher metabolism of irradiated cells.
We can deduce that the stimulatory effect we have observed under irradiation is an indirect effect of the radiations on the cells and that it might be physiological consequence of prior free radicals scavenging.
- In an attempt to understand the radiation-induced mitotic stimulations, we have initiated studies of the energetic metabolismof these cells through the metabolic way linked to the glucose consumption. Glucose uptake (Fig.3) appears now and again enhanced under chronic irradiation. However, the study of enzyme activities of glucose-6-phosphate dehydrogenase and of pyruvate kinase do not show an evident correlation between the cell growth stimulation and the level of enzyme activities.

Cell concentration at the start of culture	Daily relative variations of growth rate DAYS		
	1	2	3
150.000 ± 10.000	- 3.3 - 4.6 - 6.4 • - 2.5	- 4.6 - 9.9 • - 7.1 • - 6.6 •	- 3.2 - 16.0 • - 7.6 • - 6.7 •
220.000 ± 20.000	- 0.5 + 4.1 - 2.1 + 0.1	- 0.8 + 4.5 - 0.5 - 3.3	+ 0.5 + 1.7 - 3.0 + 6.3

Fig. 1 : Influence of the inoculation cell density on the relative variations of growth rate of cells growing in control or pre-irradiated medium. Human lung fibroblasts were inoculated in 35 mm culture dishes at the density of 15 or 22.10^4 cells. 24 hours later, the medium was removed, half of cell cultures was fed with control medium, half was fed with the same medium but pre-irradiated (5 mGy). The growth was examinated through 3 days on 8 cultures/days (4 control, 4 treated).

Daily relative variations of growth rate DAYS		
1	2	3
- 17.5 • - 4.4 • - 0.1 - 2.4	- 9.5 • - 8.7 • - 3.3 - 2.6	- 5.0 • - 9.7 • - 6.1 • - 3.5

Fig. 2 : Daily relative variations of growth rate of fibroblasts that have been exposed to supernatant of control or irradiated quiescent cell cultures. Control and irradiated cells were grown to confluence. Two days after becoming confluent, the old medium was replaced with fresh medium containing 0.1% SVF. These media (control and irradiated) were removed 3 days later, supplemented with 2.5% SVF and transferred on 24h control culture.

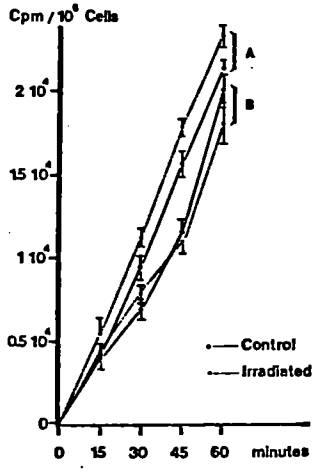


Fig. 3 : Effect of irradiation on the uptake of ^{3}H deoxyglucose into fibroblasts. A serie of Petri dishes (35 mm) with irradiated or control cells were washed in PBS Dulbecco. Cells were then flooded with 1 ml of PBS containing 1µ Ci of ^{3}H deoxyglucose and incubated at 37°C. After appropriate time, cell monolayers were rinsed with PBS, trypsinized, and aliquots were taken for liquid scintillation counting.

Cell density at the start of the experiment	Daily relative variations of growth rate Days			
	2	3	4	5
5.10^5 cells/ 25 cm^2 flasks	- 19.1• - 6.9• + 1.0	- 9.1• - 10.3• - 1.5	+ 4.1 - 6.6• - 12.8•	- 7.3 - 5.1 - 6
1.10^6 cells	+ 10.6• + 4.4 + 27.1• + 19.2•	- 5.3 + 7.5• - 6.1 + 10.5•	- 2.6 - 11.5• - 7.2• + 4.5	- 5.0 - 2.5 + 0.9 - 3.1
	log phase		deceleration phase	

Table 1 : Daily relative variations of growth rate of irradiated and control fibroblasts in relation with the cell density at the start of the experiment.

IV. Objectives for the next reporting period:

Recent data seems to show that irradiated or control quiescent or serum deprived cells are arrested in different metabolic states. So, experiments will be performed to know wether there are two separate G1 arrest points and/or wether kinetic cells entry into S phase is modified. On the other hand, investigations will be performed on the radiation-induced repair.

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

Prof. Dr. F. STEINHAUSLER
Universität Salzburg, Institüt für allgemeine Biologie,
Biochemie und Biophysik
Hellbrunnerstr. 34
A-5020 SALZBURG AUSTRIA

VI. Publications:

Title of the project no.: Subproject n° 1b :

Biophysical studies on the effects of very low doses of ionizing radiation on cells.

Head(s) of project:

Prof. Dr. F. STEINHAUSLER

Scientific staff:

Dr. B. Reubel

I. Objectives of the project:

The validity of different models for extrapolation of dose effect curves from high-level to low-level dose areas is questionable. The aim of the present investigation is therefore to give more detailed information about the reaction of cells in the low dose area using in vitro cultures. As biophysical parameter of investigations served the transmembrane resting potential (TMRP). The membrane functions as primary target for all exchange processes between cell cytoplasma and cell environment and, on the other hand, as secondary target for intracellular physiological or genetic changes. The influences of stress on the cell, such as ionizing radiation, and changes within the cell as caused by physiological parameters (e.g. cell age) can be demonstrated quantitatively by the alteration of the TMRP.

II. Objectives for the reporting period:

In the first part of this project measurements on human embryonic lung cells at different ages and levels of development, irradiated with dose rates of 0.15 - 0.84 mGy/day, and control measurements were carried out.

III. Progress achieved:

1.- Methods

Material
Human embryonic lung cells (HEL) and skin fibroblasts were cultivated as monolayer. From the primary cell strain subcultures in different strain ages (doubling time) were prepared containing a heterogeneous cell population with cells in different phases of the cell cycle.

Cultivation Method
Hel-cells and skin fibroblasts were cultivated in Eagles MEM with Hepes Buffer 20 mM + 10% foetal bovine serum + 20 µ g/ml gentamycin. The cultures were incubated at 37 degree C in an atmosphere of 21% oxygen and 0.03% CO2.

TMRP-measurements
The measuring chain is composed of a glass-microelectrode with a tip diameter of 0.1 - 0.5 µm, an electrical resistance of > 20 MOhm and filled with 3M KCl, of an electrode holder, a preamplifier, a neuroprobe amplifier and a reference electrode. The data were recorded on a strip-chart recorder and on a storage oscilloscope; optical facilities are an inverted microscope and a camera - monitor - video - system.
The impalement of the electrode into the cell is carried out with the aid of an electrically controlled micromanipulator under microscopical observation.

Evaluation
TMRP data of at least 30 single cell measurements are summarized as (geometrical) mean and (geometrical) standard deviation in the form of histograms. The comparison between data groups is carried out using the two-sided distribution-free U-test with a level of significance of $\alpha = 0.05$.

2.- Results

HEL cells:
Altogether 1400 single cell measurements have been carried out. One day before the onset of the measurements the cells were trypsinized for further cell growth from lag- to plateau-phase.
It could be shown that HEL cells (doubling time: 36), preirradiated with a dose of 12 mGy (dose rate: 0.15 mGy/day) and than consequently irradiated with a dose rate of 36 µGy/day during the measurements, increased their mean TMRP values statistically significant on the 10. day after the onset of irradiation as compared to the control values (Fig.1). No important change of the TMRP could be found 1 - 7 and 15 - 21 days after the onset of irradiation. The TMRP of HEL cells, which were preirradiated with a dose of 3.75 mGy (dose rate: 0.15 mGy/day) and then irradiated with a dose rate of 36 µGy/day, showed no significant alteration (Fig.1).
HEL-cells (doubling time:20), irradiated with dose rates of 0.84 resp. of 0.30 mGy/day, were measured in the period of 1 to 8 days after the onset of irradiation. The doses ranged from 0.3 mGy to 6.7 mGy. No statistically significant change of the TMRP of the irradiated cells as compared to the control values could be found (Fig.2).

Skin-fibroblasts:
Altogether 800 single measurements have been carried out. One day before the onset of the TMRP measurements the cells were trypsinized.
Skin-fibroblasts, irradiated with a dose rate of 0.3 mGy/day (dose: 2.1 - 2.7 mGy), were measured in the period of 7 to 9 days after the onset to irradiation. It could be demonstrated, that the mean TMRP values of the fibroblasts with radiation doses of 2.4 and 2.7 mGy are statistically significantly increased as compared to the control values (Fig.3).

Fig.1: Relative change of TMRP values (in percent of the control value) of HEL-cells, preirradiated with doses of 12 and 3.7 mGy and irradiated with a dose rate of 36 μGy/day

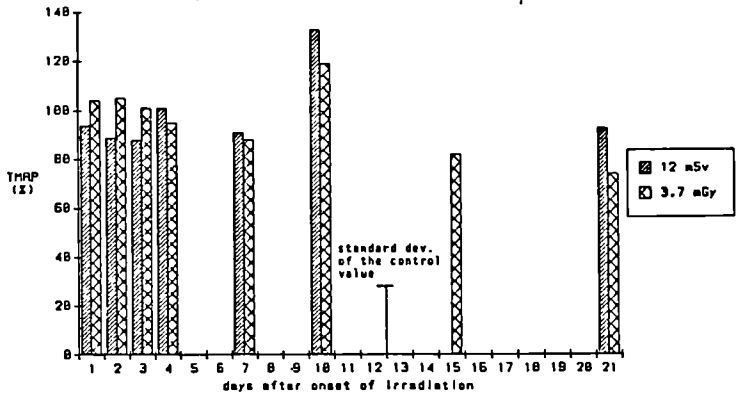


Fig.2a.: Relative change of TMRP values of HEL cells, irradiated with different doses, in percent of the control value
(dose rate 0.3 mGy/day)

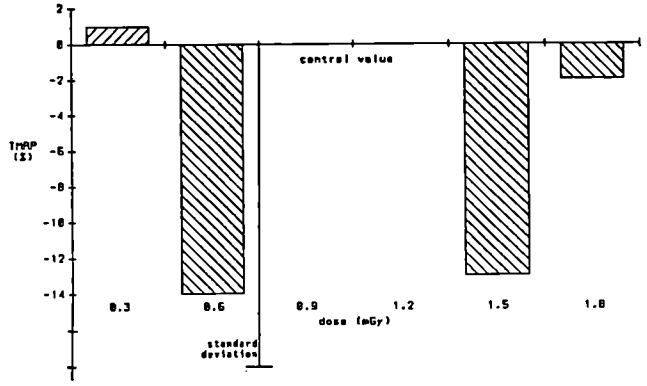
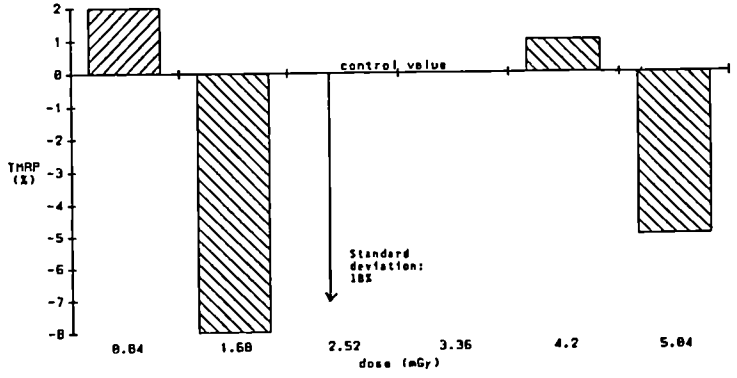


Fig.2b.: Relative change of TMRP values of HEL cells, irradiated with different doses, in percent of the control value
(dose rate 0.84 mGy/day)



Skin-fibroblasts with a strain age of 26 (doubling time) and in the plateau phase state, which were preirradiated with a dose of 4.8 mGy (dose rate: 0.15 mGy/day) and irradiated with a dose of 36 µGy/day during the experiments, reacted on the 4. - 7. day after the onset of irradiation with a statistically significant increase of the TMRP of 100% over the control values (Fig.4).

3- Conclusions

A clear effect of irradiation on the TMRP of HEL cells with doses of 0.3 - 12.7 mGy could not be detected. The TMRP increase on the 10. day after trypsinisation (preirradiation with a dose of 12 mGy and irradiation with a dose of 0.36 mGy) is not correlated with the confluency of the cell cultures (see first report) and appears as a single and irreproducible result.

The TMRP of skin-fibroblasts, preirradiated with a dose of 4.8 mGy and irradiated with a dose rate of 36 µGy/day, reacts sensitively to chronic irradiation in the confluent state, where physiological activity of the cell is reduced. A possible radiostimulation of the TMRP values of cells in the late log-phase growth could be induced by doses of 2.4 - 2.7 mGy.

Fig.3.: Relative change of TMRP values of skin fibroblasts, irradiated with different doses, in percent of the control value (dose rate 0.3 mGy/day)

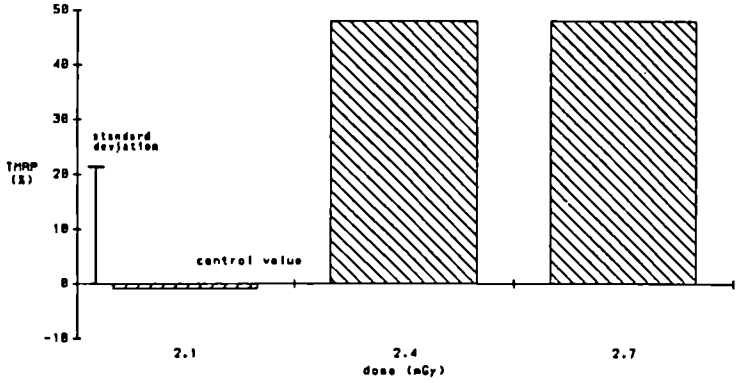
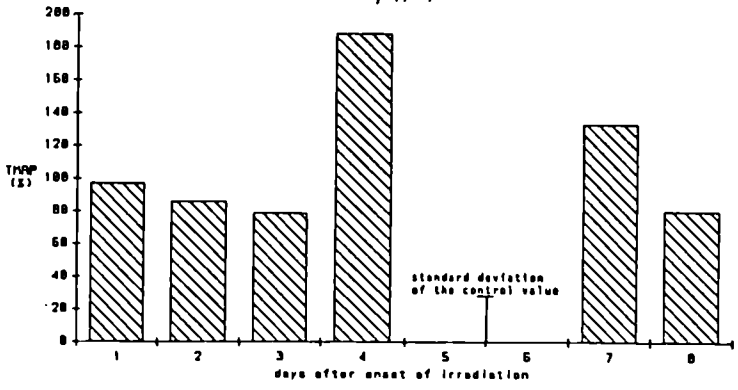


Fig.4: Relative change of the TMRP values (in percent of the control value) of skin-fibroblasts, preirradiated with doses of 4.8 mGy and irradiated with a dose rate of 36 µGy/day



Title of the project no.: Subproject n° 1c

Biochemical studies on the effects of very low doses of ionizing radiation on Cyanobacteria : Synechococcus lividus.

Head(s) of project:

CONTER A.

Scientific staff:

MURAT M.

JOURDAN R.

I. Objectives of the project:

Study on the influence of the pre-irradiation of medium and on the effects of addition of scavengers and radioprotectors on the growth of the cyanobacteria Synechococcus lividus.

II. Objectives for the reporting period:

Investigations in order to define the effects of the addition of Butanol (scavenger of $OH^{\cdot}$) oxidized or reduced glutathione, oxidized or reduced dithiotreitol, histidine or leucine, on the effects induced by the pre-irradiation of medium at 0.16 mGy a stimulating dose or at 8.20 mGy, an inhibiting dose.

III. Progress achieved:

Introduction :

We previously reported that the pre-irradiation of medium at 0.16 and 8.20 mGy before inoculation led respectively to a stimulatory or inhibitory effect of the growth of Synechococcus (next report). We also indicated that addition of catalase was followed by the disappearence of the stimulatory or inhibitory effect, while superoxide dismutase addition could not modify the effects on the pre-irradiated medium : these results indicated that H_2O_2 was involved in observed effects. We now assayed the effects of addition of some radioprotectors : the Butanol (scavenger of $OH^{\cdot}$), oxidized or reduced glutathione, oxidized or reduced dithiotreitol, histidine or Leucine.

Methodology :

Organism and culture conditions

Synechococcus lividus strain 6716 from the Institut Pasteur collection was used. Stock cultures were grown in liquid medium at a temperature of 39°5 ± 0.1°C under a continuous 700 Lux fluorescent light.
Medium : Liquid medium (Kratz and Myers, 1955) was prepared and divided in three parts : shielded or irradiated : at the two doses 0.16 and 8.20 mGy previously determined to led to stimulatory or inhibitory effects on growth. Each medium was simultaneously inoculated with cells selected in late exponential phase at a final concentration of 3.10^6 cells ml^{-1}, and distributed in ten 100 ml Erlenmeyers flasks, each filled with 50 ml of culture. Cultures were placed in a water bath and stirred at 39°5 ± 0.1°C. The illumination was 1100Lux ± 25 at the level of the cultures.

Scavengers addition

Butanol 0.1 M, oxidized or reduced glutathione (Sigma), oxidized or reduced dithiotreitol, histidine and L. leucine were added at 20 µg/ml at the inoculation.

Estimation of growth

Protein and Chlorophyll a contents were assayed in cultures checked at the 7th day. Protein content was evaluated by a Lowry procedure (1951) using serum albumine bovine (Sigma) as standard. Chlorophyll a was extracted in 90% methanol a 4°C and expressed in µg ml^{-1}, using the extinction coefficient given by Marker for k = 76.07 at 665 nm. Synthesis rates were calculated applying the formula : content at the 7th day - initial content /7.

Results

Addition of 0.1 M Butanol at the inoculation in shielded or irradiated media, led to a disappearence of the stimulatory effect produced by 0.16 mGy irradiated medium (Fig. 1). Opposite to that, addition of 0.1 M Butanol did not counteract the inhibitory effect produced by 8.20 mGy irradiated medium.
Addition of oxidized or reduced glutathione greatly increased the growth and led to a disappearence of inhibitory or stimulatory effects (Fig. 2). Addition of oxidized and reduced dithioreitol (Fig. 2), histidine or Leucine (data not shown) did not modify the radioproduced effects.

Discussion

The results indicated that the radical OH' scavenged by butanol was involved in the mechanism of the radiostimulation, but was not responsible of the inhibition of the growth. On the other hand, oxidized and reduced glutathione seemed to be good radioprotectors against radioproduced radicals in medium while either dithiotreitol, histidine or leucine were not.

References

KRATZ W.A., MYERS J., 1955, Am. J. Bot. 42, 282.

LOWRY A.H., ROSEBROUGH N.J., RANDALL R.J., 1951, J. Biol. Chem., 193, 265.

MARKER A.F., 1972, Fresh Water Biol., 2, 361.

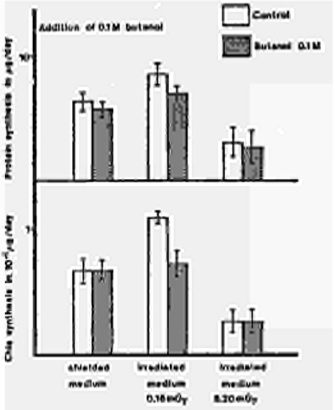


Fig. 1 : Addition of 0,1M butanol on protein and chlorophyl a synthesis at the 7th day in cultures grown in shielded or irradiated media (0,16 and 8,20 mGy). Each value is the mean ± confidence interval of 6 experiments.

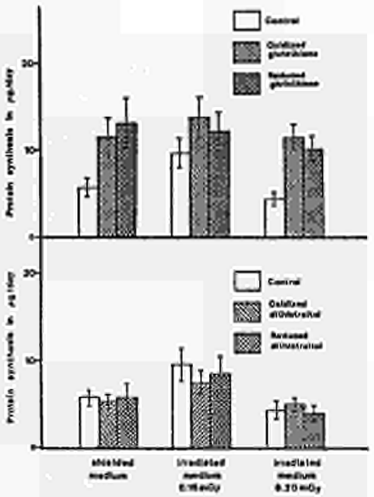


Fig. 2 : Addition of oxidized and reduced glutathione, oxidized or reduced dithiotreitol on protein synthesis at the 7th day in shielded or irradiated media (0,16 and 8,20 mGy). Each value is the mean of 4 experiments ± confidence interval.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
L F Alexander

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:

Using techniques and models reported on previously, the 1987 objectives were:

(a) Measurement programme on human volunteer subjects who had known quantities of Cobalt 57 in their lungs (see Project 2). Comparing the observed counts from these subjects over the energy range, 120 KeV, with the predicted system response as calculated from computer modelling and from lung phantom work.
(b) Refinement of computer models based on the above Cobalt 57 data.
(c) Application of refined models to the lower energies of 17 KeV (Plutonium L X-rays) and 60 KeV (Americium 241 gamma emission). Comparison of models with observed phantom responses for distributed sources.
(d) Application to man - suspected of having measurable lung contents of Plutonium and Americium.

III. Progress achieved:

1 Methodology

Previous reports have given details of the hardware, phantoms and computer modelling which have been developed during this project.

For most of 1987 one of the detector arrays, that of small square phoswiches, was out of commission due to technical faults in the pulse analysis circuitry. Most of the results described below were therefore obtained on the alternative array of larger circular phoswiches. Some extra modules have been introduced into the computer programming; these modules mainly deal with modelling build-up factors, the effects of the rib cage and modified geometric arrangements of the detectors.

2 Results

Previous work has demonstrated satisfactory agreement between observed and predicted responses at 17 KeV, Plutonium L X-ray energies, but poorer agreement at 60 KeV, Am-241 gamma energy. This observed data was accrued from a series of studies using distributed sources in a realistic Chest Phantom (Lawrence Livermore Laboratories). The measurements on phantoms and subjects containing Cobalt 57, at 120 Kev principal gamma energy, were intended to develop computer modelling so as to obtain satisfactory agreement of 120 KeV and to apply the modified models to the lower energies. Progress throughout 1987 is summarised in the Tables below, together with explanatory footnotes.

Table 1 - Phantom Work with Point Sources of Plutonium 239
Comparison of Computer Predicted Response to Observed Response as Ratio

	Left Array	Right Array	Full Array
Left Lung	1.5	0.5	1.4
Right Lung	0.6	1.0	1.0

Notes: 1 Computer models as refined by Co-57 studies.
2 Variability of point source response is very marked with Pu-239.

Table 2 - Phantom Work with Homogeneous Sources of Americium 241 and Differing Chest Wall Overlays
Comparison of Computer Predicted Response to Observed Response as Ratio

Chest Wall Thickness (cm)		Pu-239 Lungs		Am-241 Lungs	
Left	Right	L Array	R Array	L Array	R Array
0.63	0.54	1.06	0.72	0.6	0.5
0.89	0.78	0.96	0.82	0.7	0.4
1.13	1.04	1.06	0.85	0.6	0.5
1.33	1.34	1.00	0.78	0.6	0.5
1.61	1.49	0.87	0.83	0.6	0.5
Mean		0.99	0.80	0.6	0.5

Notes: 1 Using latest generation of computer models.

Table 3 - Phantom work with Distributed Cobalt 57 point sources
Comparison of computer predictions observed responses and as a ratio

Array	Basic Model		Modified(2D)		Modified(3D)		2D + Build-up	
Lung	Left	Right	Left	Right	Left	Right	Left	Right
L Lung	0.78	3.23	0.63	1.35	0.84	0.82	0.87	1.03
R Lung	2.27	1.12	1.34	0.61	1.04	0.81	1.07	0.84

Notes: 1 Each response/prediction is mean of at least ten point source distributions. Errors on each ratio are about 20%
2 Application of build-up to 3D model was not attempted.
3 Overall accuracy of method assessed as 0.93 $\pm$ 0.18.

Table 4 - Human Subjects with Co-57 - Unknown Distribution
Comparison of Computer Predicted Response to Observed Response as Ratio

Subject	Left Array	Right Array
1	0.85	1.00
2	0.97	0.98
3	1.08	1.16
Mean	0.97	1.05

Notes: The variations of the ratio with time, were presented in last year's report. Such variations have been largely removed by current computer models.

3 Discussion

1 The above summaries of results indicate that in computer modelling of response from Pu and Am responses from the Livermore phantom we have a consistent under-prediction from the right hand phoswich arrays. This would appear to arise from modelling errors, specific to the phantom, rather than technical problems and does not appear in point source studies nor in Cobalt measurement in man.

2 The use of Co-57 based models and experimentally determined Am-241 build-up factor result in an under-prediction for overall Am-241 response. This under-prediction (40%) should be compared with previous over-prediction of the order of 80% without the use of the Co-57 based models. The problem is still under investigation.

3 Studies on man, with Pu-239 and Am-241 are now necessary and are planned for the current year using subjects with small but measurable quantities of accidentally inhaled material should such subjects be available.

IV. Objectives for the next reporting period:

1 Resolution of outstanding inconsistencies with Am-241 modelling.

2 Confirmation of modelling procedures using the alternative phoswich array.

3 Measurements on man for Am-241 and Pu-239.

4 Publication of final report on 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:

"The Prediction of Detector Efficiencies in Lung Monitoring" - L F Alexander, AEEW - RSD/TM 87/8.

Quarterly Progress Reports to RPR Core Programme Committee.

Title of the project no. 2

Lung Models

Head(s) of project:

D Ramsden
P P Foster

Scientific staff:

I Pearman
M E D Bains

I. Objectives of the project:

The overall objective of this Project is to supply data and interpretation which will further lung model development.

In 1986 Winfrith participated via EULEP in an inter-species comparison of lung clearance involving seven European Laboratories. The objective of the study was to test the hypothesis that, whilst mechanical transport of particulates from the lung was species-dependent, the transport of solubilised material was species-independent. To test this hypothesis standardised monodispersed cobaltous oxide particles were inhaled by nine species (strains). Winfrith participated in the studies on man.

In association with the overall project, observation on men, containing small but measurable quantities of plutonium continued and the clearance was compared with that predicted by current lung models.

II. Objectives for the reporting period:

(a) The time-scale for the inter-species study was eight months. Measurements in man continued, outside this time-scale, whilst Co-57 levels in the lung were still above the limits of detection and whilst excretion levels were still elevated. On three of the four subjects measurements were possible throughout 1987.
(b) The preparation of the joint report on the project, planned for late 1987, has been delayed until early 1988 to incorporate all data from all laboratories. Two Workshops were held by the participating laboratories.
(c) Further measurements and lung modelling continued on one human subject with measurable quantities of plutonium in his lung.

III. Progress achieved:

1 Methodology

(a) The levels of Cobalt 57 in the lung, in urine and in faeces in the four volunteer subjects were low, as a result of both radioactive decay and earlier clearance. Counting times on the subject, using standard Whole Body Monitor techniques were long (approx 1hr). Full radiochemical analysis was necessary on all excreta samples. With careful techniques results were obtained on three of the four subjects up to December 1987.

(b) Figures 1 to 3 show comparisons of average retention/excretion with the adjusted ICRP 30 model. It is recognised that the Co_3O_4 particles were chosen to give an expected behaviour intermediate between the Class Y and Class W ICRP 30 models. This was particularly true of the expected urinary excretion functions. The observed behaviour of the 'average subject' is compared to both Class W and Class Y behaviour as modelled by ICRP 30. Both particle sizes from the ICRP 30 model are illustrated in the figures although no significant difference was predicted. Retention in the subjects was broadly what would have been predicted from Class Y behaviour in the model except there was no evidence of a one-day pulmonary clearance component for the 'average' subject.

2 Results

Typical results are shown graphically in Figures 1 to 3.

Fig 1 Lung Retention Observed (as Fraction of Initial Deposition averaged for all four subjects) Plotted Against ICRP 30 Model (Classes W and Y)

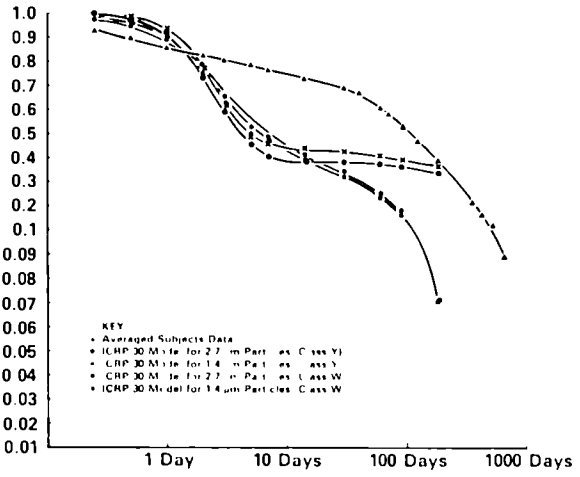


Fig 2
Daily Faecal Excretion as a Fraction of Deposition (averaged for all four subjects) Plotted Against ICRP 30 Model (Classes W & Y)

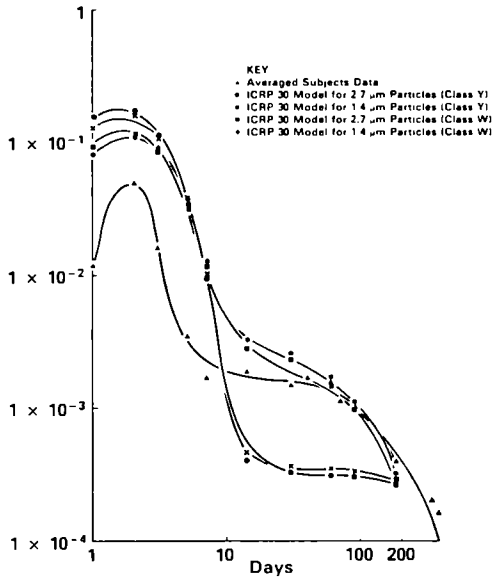
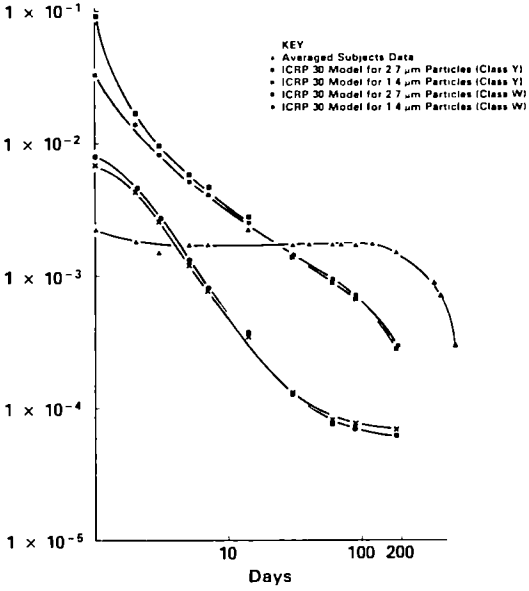


Fig 3
Daily Urine Excretion as a Fraction of Deposition (averaged for all four subjects) Plotted Against ICRP 30 Model (Classes W & Y)



3 Discussion

The results are presented in this report as fractions of initially retained material appearing in excreta or being retained in the lung. The published data for the joint study will be presented in terms of mechanial transfer fraction and solubilised fraction. The method of deriving such fractions will be discussed in the final report.

IV. Objectives for the next reporting period:

1 Publication of EULEP joint study in Spring 1988.

2 Completion of measurements on man in Spring 1988.

3 Publication of first report on Co-57 in man - late 1988.

4 Publication of report on Plutonium in man - late 1988.

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

GSF	Neuherberg, FRG
NRPB	Chilton, UK
CEA	Bruyeres-le-Chatel, F
MRC	Harwell, UK
IGT/KFK	Karlsruhe, FRG
AERE	Harwell, UK
CEA/JIC	Karlsruhe, FRG

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:

Quarterly Progress Reports to RPR Core Programme Committee.

Title of the project no.: 3

In-vitro Solubility Studies

Head(s) of project:

D Ramsden
P P Foster

Scientific staff:

I Pearman

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.

II. Objectives for the reporting period:

The experimental phase was planned to end in 1986 but decommissioning was postponed until the question of whether late bacterial growth (Pseudomonas aerginosa) had affected the solubility of the material. This question was resolved in 1986/87 and the rigs were finally decommissioned in January/February 1987.

Radiochemical analysis of all components of the three rigs should be completed within 3 to 5 months with the complete project being finished during 1988.

III. Progress achieved:

1 Methodology

Dismantling of the three rigs, held in a glove box with an inert nitrogen atmosphere, was done using standard techniques. All contents and components were separately stored and labelled for subsequent radiochemical analysis.

Radiochemical analysis of contents and components required a combination of wet and dry ashing, filtration and volume reduction. Chemical yield were determined by the addition of tracers. Isotopic analysis was done by ion-exchange resin separation and by alpha spectrometry.

Mathematical analysis of the results has concentrated on the plutonium isotopes, the uranium isotopes and on Americium 241. The patterns of fraction removed (solubilised) with time are analysed initially in terms of simple mixed exponential clearances.

2 Results

The problem of bacterial growth was never satisfactorily resolved. Testing of the membranes with colloidal and ionic gold showed changes in 'transfer characteristics' but not of sufficient magnitude to explain the step changes observed at about 900 days. There was no evidence of plutonium activity associated with the bacterial growth.

Major refurbishment of the radiochemical analytical laboratories during the year meant that the backlog of radiochemical analysis associated with this project could not be completed. There are still some 60 outstanding samples to analyse. The trends in analysis are already apparent and were reported during 1987. These are:

(a) Of the three rigs, the one containing transferrin exhibited different behaviour in the amplitudes, but not the rates, of clearance for plutoniums and uraniums. The clearance of americium was not affected by transferrin.

(b) The two rigs, not containing transferrin, showed similar solubilisation patterns and, assuming confirmation by the final activity balance, the data will be amalgamated.

(c) The fraction of the original material 'solubilised' over the three year period was of the order of:

Americium	5% with and without transferrin
Plutonium	1% without transferrin and 5% with transferrin.

(d) The uranium showed a rapid solubilisation of up to 5% (over the first twenty days).

(e) The early solubilisation component for uranium was enhanced by the presence of transferrin.

(f) The pattern of solubilisation (americium and plutonium) could usually be summarised by two components, one of intermediate half time (approximately tens of days) and one of long half time (approximately hundreds of days).

(g) The evidence so far is not inconsistent with ICRP Class Y behaviour.

3 Discussion

Full analysis of the three year's data is continuing. The trends noted above will be quantified as solubilisation rates and amplitudes. These expressions can then be compared with the expressions currently available for the dosimetric interpretation of urinary excretion. They can also be compared with the experimental information available from the Co-57 studies in man and with observed urinary clearance of plutonium in man.

IV. Objectives for the next reporting period:

The Project will be completed leading to full publication of the results.

The data will be compared to current lung models and to urinary clearances observed in man.

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:

"Solubility Studies on an Industrial Plutonium Dust" - P P Foster, I Pearman, M E D Bains, D Ramsden.

The Design and Interpretation of Inhalation Studies - Hanover, March 23-27 1987.

Quarterly Progress Reports to RPR Core Programme Committee.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
Y.Q. CHEN
M. BRUNFAUT

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. Selective conditions were sought for the isolation of partial transformants from cultures of human epidermal cells, with the object of quantifying phenotypic alterations which are induced by radiation and contribute to malignant transformation.
b. More effort was devoted to the characterization of radiation-induced recovery and mutator phenotypes in cells from ataxia telangiectasia patients, using viral single- and double-stranded DNA probes for the determination of time-courses and dose effects.
c. Molecular changes associated with steps in radiocarcinogenesis were investigated by comparing a normal human fibroblast strain and its gamma ray-transformed derivative for their ability to express specific genes of oncotropic parvoviruses.

III. Progress achieved:

Main progress achieved during the last year can be summarized as follows :

a. Development of a human keratinocyte system for quantifying the induction of intermediate stages of transformation by ionizing radiation.

Well-controlled culture conditions have been defined, allowing the identification and isolation of human epidermal cells expressing phenotypic alterations which contribute to malignant progression and complement the action of specific oncogenes.

b. Characterization of a human cancer-prone condition associated with radiohypersensitivity.

The previously reported deficiency of fibroblasts from ataxia telangiectasia patients for a conditional recovery response to X-rays, was extended to a mutator phenotype which is normally activated in X-irradiated human cells and can be revealed with a herpes virus probe.

c. Analysis of gamma-ray transformation of human fibroblasts.

Molecular changes underlying the phenotypic alterations which are induced by ionizing radiation and commit human cells into the process of malignant transformation, were probed by means of oncotropic parvoviruses. In vitro transformation of human fibroblasts by gamma-rays was found to correlate with the activation of an expressor phenotype leading to the enhanced production of parvoviral non-structural proteins and the exacerbation of viral cytopathic effect.

a. In vitro transformation of human keratinocytes

Cells established from various human squamous cell carcinomas are resistant to terminal differentiation stimuli and exhibit reduced growth factor requirements compared with normal keratinocytes. In vitro culture conditions were defined, which allow the quantitative measurement of the latter properties. Using a synthetic medium supplemented with defined growth factors and feeder layers of lethally gamma-irradiated mouse cells, clonal growth of human keratinocytes could be determined as a function of above-mentioned parameters. The potential use of this system to quantify the induction of partial transformation of human cells was validated by comparing growth parsmeters of normal human epidermal cells and of a spontaneously established line of human keratinocytes. Among the conditions which caused growth arrest of normal keratinocytes but permitted the replication of the transformed derivative, were elevated levels of calcium, the addition of the tumor promoter TPA and reduced growth factors concentrations. Interestingly, the established line was susceptible to further oncogenic transformation by an activated human ras oncogene, whereas normal human keratinocytes were not. Thus, this system can now be applied to the measurement of phenotypic alterations which may be induced by radiation and contribute to oncogenic transformation of human epithelial cells in cooperation with oncogenes.

b. Conditional responses to radiation in cells from ataxia telangiectasia (AT) patients

Our investigation of cellular defects associated with the cancer-proneness and radiosensitivity syndrome AT was pursued. We reported previously that AT cells are deficient for a conditional process which is triggered by X-rays and denoted as Enhanced Reactivation (ER). ER takes

place when the survival of a damaged virus is higher in host cells pretreated with radiation or other agents than in untreated cells. AT cells proved to be deficient for ER of a single-stranded DNA virus (H-1) but proficient in ER of a double-stranded DNA virus (HSV-1). These observations led us to propose that the ER response involves at least two components and that AT cells may be impaired in one component of specific importance for the survival of single-stranded DNA, such as replication over a damaged template. Such a possibility was tested by measuring the capacity of AT cells for another conditional response, Enhanced Mutagenesis (EM). EM is defined as an increased virus mutagenesis in pretreated versus untreated cells. A forward mutation assay was used to measure the fraction of mutants among the progeny of intact or damaged HSV-1 produced by mock- or X-preirradiated cells of normal or AT origin. The data suggest that in normal human cells, X-rays trigger the expression of an error-prone EM process which contributes little to the survival of double-stranded DNA, and that this process may be deficient in AT cells.

c. Susceptibility of radiation-transformed human cells to oncotropic viruses.

We have previously shown that human fibroblasts immortalized and transformed by ionizing radiation can be distinguished from their normal progenitors by their greater sensitivity to the lytic action of parvoviruses H-1 and MVM. This observation was exploited to characterize molecular changes which accompany the radioinduction of an immortal and transformed, yet non tumorigenic phenotype. Gamma ray-transformed cells did not necessarily sustain a productive parvovirus infection. However, transformants had a greater capacity for specific steps of the parvoviral life-cycle. Thus, transformed human fibroblasts amplified parvoviral DNA to a 10-85 fold greater extent than normal cells. Moreover, viral transcripts and proteins were 3-4 and 12-20 times more abundant in transformed versus normal cultures. The production of parvoviral non-structural proteins has been associated with cell toxicity and amplification of viral DNA. Consequently, the overexpression of corresponding parvoviral genes in radiation-transformed human fibroblasts may provide both a clue to the selective killing of the latter cells by parvoviruses, and a marker of early cellular changes in the cascade of events triggered by radiation and leading to malignancy.

IV. Objectives for the next reporting period:

a. The susceptibility of normal human keratinocytes to transformation by X-rays will be determined. Phenotypic alterations in cellular responses to growth modulators will be monitored under chemically defined culture conditions. Dose- effect relationships will be sought for suitable transformation parameters.

b. Mutagenesis and gene expression will be measured after X-irradiation in ataxia telangiectasia cells and related controls, using viral and transient assays, respectively.

c. The involvement of radiation in cooperative effects leading to neoplasia will be studied by attempting to achieve full transformation of radiation-immortalized human keratinocytes by means for transfected oncogenes. Conversely, X-rays will be tested for their ability to complement the partial transforming effect of incomplete oncogenes on rat fibroblasts.

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, Rijks Universiteit, Leiden 2333 AL, The Netherlands (A. van der Eb)

- Department of Pathology, Kawasaki Medical School, Kurashiki, Japan (M. Namba)

VI. Publications:

- Avalosse, B.L., Chen, Y.Q., Cornelis, J.J., Duponchel, N., Becquart, P., Namba, M. and Rommelaere, J. (1987)
  Amplification of parvoviral DNA as a function of host cell transformation.
  In : "Accomplishments in Oncology", vol. 2, Eds. H. zur Hausen and R. Schlehofer, J.B. Lippincott Publ. Philadelphia, pp. 140-152.

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  In "Proceedings of the Second Congress of the European Society for Photobiology" Eds. R.H. Douglas, J. Moan and F. Dell' Acqua
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  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, in press.

- Vos, J.M. and Rommelaere, J. (1987).
Replication of DNA containing apurinic sites in human and mouse cells probed with parvoviruses MVM and H-1.
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In : "Parvovirus Handbook" Ed. Tijssen, CRC Press,Boca Raton,in press.

- Hilgers,G., Abrahams, P.J., Schouten, R.,Cornelis, J.J.,Lehmann,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 Société de Biologie, in press.

- 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., in press.

Abstract.

- Chen, Y.Q., Cornelis, J.J., Tuynder, M.C., Boukamp, P., Fusenig, N.E. and Rommelaere, J. (1987).
Transformation of human epidermal cells correlates with an increase in their susceptibility to autonomous parvovirus H-1.
Archives Intern. de Physiologie et de Biochimie, 95 (3), B112.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
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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, n. BI6-103-1.

Head(s) of project:
Prof. Giovanni B. Rossi.

Scientific staff:
Paola Rizza, Antonella Pecorelli, Simonetta Pulciani, Anna Maria Anastasi.

I. Objectives of the project:
Studies over the last few years have provided evidence that several cellular genes may have potential trasforming activity (proto-oncogenes). The proto-oncogenes have been discovered first as the cellular progenitors of retroviral transforming genes (1, 2). Thereafter, altered expression of the proto-oncogenes has been correlated with development of non-viral induced neoplasia (3). These findings prove cancer a disease derived from damage to the cell genetic apparatus.
Spontaneous as well as x-ray-induced murine tumors have been chosen as working models to investigate at a molecular level the mechanisms which may confer malignant properties to cellular proto-oncogenes.

II. Objectives for the reporting period:
Spontaneous reticulum cell sarcoma (RCS) tumors occur in 90% of SJL/J mice of 8-13 months of age (4). By DNA-mediated sequence transfer protocols (5-6) we detected the presence of a transforming gene in DNA of transplantable cell lines established from spontaneous RCS (7).
The molecular cloning and the characterization of the RCS transforming sequences may give new insights in the genetic influence on neoplasia onset and development, spontaneous as well as induced.

III. Progress achieved.

METHODOLOGY:

As described in: Pulciani et al. (1987), Cancer Research 47: 523-526.

RESULTS:

Three RCS transplantable tumor cells were injected into young SJL/J mice to allow tumor cells to grow. High molecular weight DNA has been extracted from these tumor cells and transfected into normal mouse fibroblasts, i.e. NIH-3T3 cells (8). Round foci, with an easily distinguishable morphology, could be scored in the transfected cultures. RCS transplantable tumors cells and NIH-3T3 cells are both mouse cells, therefore it is difficult to demonstrate the presence of donor DNA into the selected transformants. Tumor DNA were cotransfected with the pSV_2 neo plasmid which can be readily detected in the NIH-3T3 genetic background (9). The pSV_2 neo plasmid contains the bacterial gene for neomycin resistance under regulatory sequences which allow its expression into recipient eukaryotic cells, and enable them to grow in the presence of the drug G418. Foci obtained in the latter transfections were able to grow in presence of G418, and their DNAs were shown to contain plasmid sequences by Southern blot analysis (10). These results demonstrate that the morphological alteration seen in transfected cells was due to the acquisition of new genetic information.

Recently, the supernatant medium from in vitro cultured NIH-3T3 RCS/transfectants, induced foci of transformed cells when tested on monolayer of normal mouse and rat fibroblasts. The foci obtained in these latter experiments have similar morphology to those scored in the transfection experiments performed with RCS tumor DNAs. These experiments suggest a retrovirus as etiologic agent of the neoplastic process under analysis.

DISCUSSION:

Evidence is presented of activated transforming sequences in RCS transplantable cell lines. The latter experiments performed with the supernatants from NIH-3T3 RCS/trasfectants strongly suggest the role of transforming virus in the tested tumors.

Previous studies, directed to demonstrate the viral etiology of RCS tumors, haven't been successeful. On the other hand, genetically linked murine tumors, such as thymic limphomas of AKR mice and mammary carcinomas of C3H or GR mice, have retroviral ethiology (11). SJL/J mice contain in their DNA several endogeneous retroviral sequences and it would be interestingly to determine which of those have been activated in the tested RCS

transplantable cell lines. Thereafter, it will be important to point out the molecular mechanisms involved in their oncogenic activation.

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12. Janowski, M., Boniver, J., and Maisin, J.R. (1982). Common proviral integration sites in C57BL mouse lymphomas induced by radiation leukemia virus and absence of novel virus-related sequences in radiogenic lymphoma DNA. Leukemia Res. 6, 285-287.

IV. Objectives for the next reporting period:

Previous studies have shown the role of retroviruses in the development of thymic lymphomas of x-rays irradiated C57Bl mice (12). It will be our purpose to irradiated SJL/J mice to determine if the x-rays may induce neoplastic processes along with the expression of endogenous retroviral sequences. SJL/J mice will be irradiated with 1.5 Gy four time, weekly (6 Gy total), and checked to determine which tumors may develop. In particulary our pupose will be to investigate: a) the time required for the tumors to develop; b) the influence of x-rays treatment on the onset of RCS spontaneous tumors.

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

Dr. Benjamin Bonavida, Jonathan Kats: UCLA, Department of Microbiology and Immunology UCLA School of Medicine, Center for the Health Sciences, Los Angeles, California 90024.

Dr. Francesco Mauro, Dr. Vincenzo Cevelli, Dr. Vincenzo Di Maio, Dr. Anna Saran: PAS-FIBI and PAT, ENEA, CRE Casaccia, S.P. Anguillanese 301, Rome, Italy.

VI. Publications:

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, 523-526.

Pulciani, S., Sakamo, T., Ohnishi, K., Anastasi, A.M., Pecorelli, A., Fiorucci, G., Oppi, C., Rossi, G.B., and Bonavida, B. Detection of a transforming gene in spontaneous reticulum cell sarcoma of SJL/J mice. A genetically linked and host-dependent neoplasia. UCLA Symposia on molecular and cellular biology, 16th annual meeting, January 17-February 5, 1987 (Abstract).

Anastasi, A.M., Pecorelli, A., Pulciani, S., Di Lonardo, A., Ohnishi, K., Sakamo, T., and Bonavida, B. A transforming gene in spontaneous reticulum cell sarcoma. International congress cancer metastasis biological and biochemical mechanisms, Bologna, May 13-15, 1987 (Abstract).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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Biomedical Effects Department
NRPB
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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
Miss 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 examine the lung clearance of uranium trioxide (UO_3) obtained from the reprocessing of nuclear fuels.

3. To investigate the efficacy of DTPA and or LICAM-C on the decorporation of Pu and Am present as different chemical forms.

III. Progress achieved:

Methodology

The new 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. Studies in progress include those on the comparative metabolism of ^{239}Pu and ^{241}Am after inhalation of $^{239}PuO_2/^{241}AmO_2$ and residues obtained from a nuclear weapons test site; and on the behaviour of actinides and fission products in residues obtained from a cooling pond. 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 $^{239}PuO_2/^{241}AmO_2$ by rats and guinea pigs, the increase in the Pu/Am ratio of the material retained by the lungs was less than 3% at 168d. This study will subsequently include data on the transportability of the radionuclides. After the inhalation of test site material, the Pu to Am ratio in the lungs of rats and guinea pigs increased by about 3% at 28d and in the lungs of rats by about 30% at 168d. Lung retention data on guinea pigs will concentrate mainly on the interval from 1 to 4 years after exposure. The rate of translocation of ^{239}Pu from the lungs to blood in rats between 0 and 28d and 28 and 168d respectively were 0.31% and 0.17% d^{-1}. The corresponding values for ^{241}Am were 0.41 and 0.17% d^{-1}. These values suggest that this material more closely resembles that of a class W compound than a class Y compound. The relationship between the systemic content and cumulative urinary excretion of ^{239}Pu during the first 28d after exposure was similar to that obtained after the intravenous injection of ^{239}Pu citrate. Both studies will follow retention for the animals lifespan before the ALI's and limitations on the use of ^{241}Am for interpreting chest monitoring data are assessed.

Studies have continued on the mechanisms involved in the lung clearance of ^{239}Pu and other radionuclides present in industrial dusts and residues. Their response to DTPA treatment is considered to be an appropriate method of obtaining such information. When a suspension of a residue obtained from a cooling pond at a nuclear facility was instilled into the lungs of rats, the amounts of ^{239}Pu, ^{241}Am, and ^{144}Ce present in the lungs and other body tissues at 28d were all about 45% and 4% respectively of the initial deposit. After treatment with DTPA (30 μmol kg^{-1} administered ip 30m, 6h, 1,2,3d and then twice weekly to 26d) the lung and tissue contents were about 90% and 30% of the control values above. These data contrast markedly with those obtained with DTPA treatment after the administration of Pu and Am as their nitrates; in this case the lung and tissue contents were reduced to about 1% and 3% of their control values. The results suggest that the residues do not contain appreciable amounts of the actinides as their transportable forms and that transfer to blood occurs as a result of the slow dissolution of the particles entrained, presumably, in alveolar macrophages. It is also noteworthy that

DTPA did not have an appreciable effect on the ratio of Pu to Am or Ce retained in the lungs.

Previously, animal experiments have suggested that ^{241}Am can be used for the assessment of the lung content of ^{239}Pu in humans. However in some industrial situations eg. during the handling of irradiated fuel, the identification of ^{241}Am may be difficult due to the excessive amounts of fission products present. The lung retention kinetics of ^{238}Pu, ^{241}Am and ^{139}Ce in the rat have been found to be similar after intratracheal injection as their nitrates eg. 15%, 21% and 17% respectively of the initial deposits were retained in the lungs at 126 days after exposure. These results suggest that the assessment of ^{239}Pu in the lungs from measurement of ^{144}Ce would be of potential benefit in the above industrial situation. Further studies are in progress to substantiate this possibility.

A study on the efficacy of a polymeric form of LICAM-C for eliminating ^{238}Pu from the rat after its inhalation as the nitrate showed that it was substantially inferior to DTPA for this purpose. Using the same regimen as described above for DTPA, the lung content was reduced to only 36% of the control value (cf ~1% for DTPA). After IV injection of ^{238}Pu as the citrate, both compounds were equally effective. A pure form of LICAM-C has now been obtained and the inhalation experiment described above will be repeated.

An experiment has commenced recently to investigate the transportability of UO_3 obtained by the thermal degradation of uranyl nitrate. Seven days after the intratracheal instillation of the respirable fraction of the dust (<5μm Stokes' diameter) into the lungs of rats, more than 60% of the initial deposit of uranium (27 μg) translocated to the blood and about 10% was retained by the lungs. The rate of transfer to blood during this interval, 22% of the lung content per day, indicates that the compound should be assigned to inhalation class D and intakes by workers limited to 2.5 mg d^{-1} on the basis of chemical toxicity. The results so far are in good agreement with those obtained for other UO_3 preparations. The study will continue for 3 months.

IV Objectives for the next reporting period:

Continuation of studies on the lung clearance characteristics of Pu and Am present in residues formed or likely to be formed in nuclear fuel process lines. Investigation of the efficacy of a pure form of LICAM-C and the chelating agent DFO-HOPO on the decorporation of Pu and Am inhaled as their nitrates. Completion of a study on the lung clearance of UO_3 obtained from reprocessing.

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

Dr. H. Metivier, 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.

VI. Publications:

A new system for administering radioactive aerosols to rodents. A. Hodgson, J.C. Moody, M.R. Bailey, J.W. Stather and H. Toivonen. in Aerosols - Their Generation, Behaviour and Applications. Proc. First Conference of The Aerosol Society, Loughborough Univ. of Technology, 31 March - 1 April 1987, pp105-108.

The clearance of uranium after deposition of the nitrate and bicarbonate in different regions of the rat lung. M. Ellender. Human Toxicol. 6 479-482, 1987.

The metabolism of uranium in the rat after inhalation of two industrial forms of ore concentrate : The implications for occupational exposure. G.N. Stradling, J.W. Stather, S.A. Gray, J.C. Moody, M. Ellender, A. Hodgson, D. Sedgwick and N. Cooke. Human Toxicol. 6, 385-393, 1987.

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 mice. To initiate studies on the ability of alpa-emitting bone-seeking radionuclides to produce ostesarcoma and myeloid leukaemias in CBA/H mice

III. Progress achieved:

Methodology

Prior to setting up a study to examine the relative toxicity of ^{239}Pu, ^{241}Am and ^{233}U in the CBA/H mouse using the induction of osteosarcoma and acute myeloid leukaemia as end points, the tissue distribution and retention characteristics of the three actinides up to 448 days after intraperitoneal administration to male and female mice are being determined. Detailed autoradiographic studies of the distribution of these radionuclides with time in two bones (femur and lumbar vertebrae) are also being carried out.

Results

The results have shown that in male mice 19.6% (Pu), 20.7% (Am) and 2.5% (U) of the initial activity was retained at 448 days. These results reflect the much faster rate of elimination of uranium than of the higher actinides from the body. Of the retained activity 86.1% of Pu, 94.3% of Am and 99.4% of U was deposited in the skeleton.

Similar results were obtained from female mice although some differences were observed. Female mice retained slightly higher levels of plutonium and americium citrate than male mice and male mice retaining slightly more uranium citrate than female mice. In both sexes the peak skeletal activity occurred at 28 days after administration of plutonium and americium citrates and at 7 days after administration of uranium citrate.

A study of the relative distribution of the radionuclides between the different bones of the skeleton is continuing.

Average bone doses to 224 and 448 days for all radionuclides have been calculated following a single injection of 40 kBq/Kg. At 224 days the average doses to bone for male mice were 0.27 Gy (Pu), 0.29 Gy (Am) and 0.035 Gy (U) and for female mice were 0.35 Gy (Pu), 0.36 Gy (Am) and 0.067 Gy (U). At 448 days the average doses to bone in male mice were 0.48 Gy (Pu), 0.53 Gy (Am) and 0.082 Gy (U) and for female mice were 0.55 Gy (Pu), 0.78 Gy (Am) and 0.102 Gy (U). From these data injection schedules for the toxicity study have been established.

The studies of radionuclide distribution within two bones (femur and lumbar vertebrae) using autoradiographic techniques are also in progress. Results are available to 224 days at present.

Discussion

The autoradiographic studies have shown that plutonium is quickly deposited onto the endosteal bone surfaces and is later taken up by macrophages in the bone marrow. Americium is deposited onto all bone surfaces including periosteal and vascular surfaces, and is taken up to a lesser extent than plutonium my macrophages. Uranium is deposited onto discrete regions of endosteal and periosteal surfaces, possibly those associated with bone turnover and enhanced metabolic activity.

The distribution of the three radionuclides within individual bones will be compared with the differences in bone dose and the sites of tumour formation.

The toxicity study of the three radionuclides is planned to commence in the spring of 1988 and will consist of three experimental groups at dose levels of ^{239}Pu of 25, 15 and 5 kBq/kg which are expected to produce tumour incidences of 40, 25 and 7.5% respectively. Using the metabolic and dosimetric data already obtained americium and uranium will be given at levels calculated to give equivalent skeletal doses. The animals will be kept for their lifespan and observed for tumour formation.

IV. Objectives for the next reporting period:

The main toxicity study will start in the spring of 1988. Levels of plutonium have been chosen which will give an incidence of osteosarcoma of 40, 25 and 7.5%. Levels of americium and uranium will be administered which will give the same average skeletal dose. Comparison of the dose-response curves for each radionuclide will establish their relative toxicity.

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:

Priest, N.D. An age-related model for the dosimetry of alpha-emitting bone surface-seeking radionuclides in man. Proc. CEC Symposium on Age-related Factors in Radionuclide Metabolism and Dosimetry. Angers, France, November 1986. p183-192, 1987.

Priest, N.D. The prediction of the relative toxicities of radium-224 and radium-226 in the bones of mice using Monte Carlo Techniques. Brit. J. Radiology 60, 677-680, 1987.

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, Mr. J. Moody

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:

Further development of techniques required to conduct the proposed studies of particle clearance from the human respiratory tract, and commencement of the experiments themselves.

III. Progress achieved:

(i) Measurement of particle clearance rates from different regions of the human respiratory tract.

During the last reporting period approval was given by the Ethics Committee and the Board to a study of the biokinetics of ^{88}Y administered intravenously to human volunteers. ^{88}Y is employed as a label for the fused aluminosilicate particles (FAP) used in long-term studies of particle clearance from pulmonary lung. The contribution to clearance made by particle dissolution is determined from measurements of urinary excretion of the label. This requires information on the retention and excretion of ^{88}Y following systemic uptake and no such information on tracer level yttrium in man is known. ^{88}Y was administered to two volunteers by intravenous injection of 0.5 ml of 0.9% sodium chloride solution to which ^{88}Y citrate at ph 7 had been added. The first subject received 0.4 kBq in a pilot experiment, the second 4.0 kBq. Total body and organ (liver, bone and bladder) retention of ^{88}Y are being measured using NaI detectors in a low-background enclosure. Quantitative faecal and urinary collections were made for 5 and 14 days respectively, and 72 hour urine samples obtained at approximately monthly intervals. A preliminary assessment of results indicates that in both subjects about 25% of the injected activity was lost with a half-time of 1 day, over 90% of it in urine. The rest was avidly retained. At 1-3 months after injection about 0.02% of the remaining activity was excreted in urine per day. The activity in the body was widely distributed, consistent with mainly skeletal deposition, but activity in the liver could be distinguished and showed a retention half-time of about 60 days.

For comparison, groups of 4 rats were intravenously injected with the same ^{88}Y solutions used in the human studies. Urine and faeces were collected for 4 days, and the animals killed to determine the tissue distribution. Generally results were similar to those for humans: about 25% of the injected activity was excreted in urine, mainly on the first day, and 5% in faeces. About 5% of the activity remaining in the body was in the liver.

Approval was given by the Ethics Committee and the Board for a human volunteer study of the deposition and clearance of inhaled particles in the nasal passage. The objectives are to determine, as functions of particle size and breathing pattern, the pattern of clearance of particles deposited in the nasal passage, ie., the fraction cleared rapidly, the retention time of particles in the front of the nose, the importance of nose-blowing as a clearance mechanism, and the degree of variation between individuals. Up to 10 subjects will inhale polystyrene or fused clay particles of uniform size labelled with ^{97}Ru, a gamma-emitting radionuclide with a half-life of 2.8 days. Retention in the nasal passage will then be followed by external counting of gamma-rays for at least 24 hours. Pilot experiments will be conducted using particles labelled with ^{99m}Tc, half-life 6 hours.

Progess has been made on developing the techniques and equipment needed to conduct the experiments. The polymer chemistry of procedures for producing monodisperse polystyrene particles labelled with ^{97}Ru has been examined and suppliers of materials identified. Currently, two approaches are being investigated. One involves producing a monomer carrying a chelating agent, selective for Ru(III), which can be polymerized once it has taken up Ru(III) and used to produce monodisperse particles; the other involves binding Ru(III), by a chelating agent, to commercially available

monodisperse particles. Apparatus to administer the labelled aerosol under the required conditions is being designed and assembled. A laser photometer to measure the particle concentration in inhaled and exhaled air is being obtained from the GSF, Frankfurt. A bicycle ergometer has been installed to permit inhalations to be carried out at breathing patterns determined by set rates of working. A detector system has been designed to enable measurements to be made of activity in the head, lungs and stomach, during and immediately after administration. Subsequent measurements will be made in the low-background facility.

(ii) Animal experiments

The studies of the lung clearance of cobalt oxide particles described under project 4 also provide information on factors affecting mechanical transport rates. The interspecies comparison of the lung clearance of 0.8 μm and 1.7 μm cobalt oxide particles has provided further support for the hypothesis that the mechanical transport rates are independent of particle composition. Because the material is moderately soluble, precise measurements of mechanical transport rates could only be made in species in which the rate is relatively high. In those species, which include the hamsters and rats used at NRPB, mechanical transport rates estimated for the two particle sizes were similar, and also similar to estimated mechanical transport rates in these animals derived from previous experiments with FAP, which dissolves in the lung much more slowly than the cobalt oxide. The new experiment in which rats of several ages inhaled cobalt oxide particles, will provide information on the effect of age on mechanical transport rates.

IV. Objectives for the next 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.

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:

Stahlhofen, W., Gebhart, J., Rudolf, G., Scheuch, G. and Bailey, M.R. Investigation of human lung clearance kinetics with inhaled pulses of radioactively labelled particles. IN Aerosols, Their Generation, Behaviour and Applications. Proceedings of the First Conference of the Aerosol Society, Loughborough, 31 March - 1 April 1987.

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
Ms. S. Gray
Mr. A. Hodgson
Mr. J. Moody

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 measurements of retention and excretion of ^{57}Co following inhalation of cobalt oxide by rats, hamsters and guinea pigs. To measure retention and excretion following intratracheal injection of cobalt. To calculate lung clearance rates in the inhalation experiments, 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:

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 continued. Most animal measurements started early in 1985, but the human experiment (at AEE Winfrith) was delayed until February 1986. To coincide with it, a second experiment on HMT rats was carried out at NRPB. This was completed 400 days after inhalation. The results were similar to those obtained in the rats exposed in 1985, so that if any changes in composition had occurred to the material in the intervening period, they did not greatly affect its lung clearrance characteristics.

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, M(t), were calculated as fractions of the activity remaining in the lungs. In the guinea pigs, rats and hamsters studied at NRPB, S(t) could be determined from about 10 to 350 days after inhalation. For the 1.7 μm particles initial values of S(t) were similar, ranging from 0.3% d^{-1} in guinea pigs to 0.6% d^{-1} in rats. In all three species, S(t) increased slowly with time to about 1% d^{-1}, reaching this value at about 150 days, 250 days and 300 days respectively in the guinea pigs, rats and hamsters. For the 0.8 μm particles, S(t) was generally higher than for the 1.7 μm particles, showed more pronounced changes with time and greater differences between species. Initial values were 0.7, 1.6 and 0.6% d^{-1} respectively in guinea pigs, rats and hamsters. All increased to a peak of about 3% d^{-1}. This peak value was reached at about 350 days in guinea pigs and 250 days in hamsters, but in rats at only 50 days after inhalation. In rats the value of S(t) fell markedly again, reaching about 0.5% d^{-1} by the end of the experiment.

To investigate the dependence of S(t) on time, a simple model of translocation to blood from particles dissolving in the lung was developed. Assuming that all particles are the same size and dissolve uniformly without changing shape; that a fraction, q, of the dissolved Co remains in the lung indefinitely while the rest is transferred instantaneously to the blood; and that the number of particles in the lung remains constant (ie. mechanical transport is neglected), then S(t) is given by:

$$S(t) \quad + \quad \frac{(1 - q) f_o \left[1 - \frac{f_o t}{3}\right]^2}{q + (1 - q)\left[1 - \frac{f_o t}{3}\right]^3}$$

where f_o is the initial dissolution rate of the particles. According to this model, when f_o is less than about 0.8% d^{-1}, little change in S(t) occurs in 6 months. For higher values of f_o, S(t) is very sensitive to the values of f_o and q. Thus there can be a steady increase in S(t); a rapid increase followed by a decrease; or an almost constant value followed by a decrease. Considering the simplifying assumptions made, the model is remarkably well able to account for the different forms of S(t) observed, including those obtained at the other laboratories participating in the study (see V).

Considerable effort was put into co-ordinating and summarisng the results from the various collaborating laboratories to produce a final report for publication. The results suggest that S(t) and M(t) are independent. Although S(t) varies much less between species than M(t), the highest translocation rate was still several times the lowest. Inter-species comparisons on other materials and studies of the mechanisms involved in translocation are needed to improve our ability to assess translocation rates for the human lung from the results of animal experiments.

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. An experimental study of the effect of age on alveolar clearance of inhaled particles was therefore initiated. ^{57}Co-labelled cobalt oxide particles were administered to rats, as it was found in the interspecies study described above that this experimental model enables both S(t) and M(t) to be measured for 6 months after intake.

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, aged 3 weeks, 12 weeks, 20 weeks and 1 year, were simultaneously exposed to an aerosol of the particles by nose-only inhalation in the new exposure system developed under this contract. Whole body activity, and faecal and urinary excretion rates are determined at frequent intervals. Four animals from each age group were killed at 7 days and 3 months to determine the distribution of ^{57}Co in the body. The remaining animals will be killed at 6 months after inhalation. A positive correlation was found between the initial lung deposit and body weight, with the 1 year old rats having 3-4 times greater deposition than the 3 week old rats. The mean whole body retention in each age group at one and three months after inhalation is shown in the table below. In all age groups more than 90% of the activity in the body was associated with the lungs at 7 days after inhalation, and more than 95% at 3 months. Measurements of excreted activity are continuing. Supplementary experiments will be conducted on animals of different ages 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. The results will be used to estimate the rate of translocation of ^{57}Co to blood from particles in the lung, and the rate of mechanical transport of particles from the lungs to the GI tract, from the measured excretion rates.

Table of results

Age	Number exposed	Whole body retention, % activity retained at 7 days (mean ± SD)	
		1 month	3 months
3 weeks	14	56.6 ± 2.6	16.0 ± 1.2
12 weeks	16	57.5 ± 1.2	20.0 ± 2.2
20 weeks	11	55.5 ± 1.6	20.3 ± 1.9
1 year	12	62.3 ± 3.0	24.1 ± 4.3

IV Objectives for the next reporting period.

To complete 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.

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

Dr. S. Andre, Dr. H. Métivier,
CEA/IPSN/SPE/STCE Bruyeres-le-Chatel, France.
Dr. G.A. Ferron, Dr. B. Haider, Dr. W.G. Kreyling,
Institut für Strahlenschutz, GSF Neuherberg, West Germany.
Dr. E. Drosselmeyer, Institut für Genetik und Toxicologie,
Kernforschungszentrum Karlsruhe, West Germany.
Dr. A. Batchelor, Dr. G. Patrick, Mrs. C. Stirling,
Medical Research Council Radiobiology Unit, Harwell, UK.
Mr. A. Black, Dr. A. Morgan, Mr. R.J. Talbot,
Atomic Energy Research Establishment, Harwell, UK
Mr. P. Foster, Mr. I. Pearman, Mr. D. Ramsden,
Atomic Energy Establishment, Winfrith, UK.
Dr. S. Pickering, CEC Joint Research Centre, European Institute for Transuranium Elements, Karlsruhe, West Germany.

VI Publications:

An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles. 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, I. Pearman, S. Pickering, D. Ramsden, C. Stirling and R.J. Talbot. Proceedings of the Second International Symposium on Deposition and Clearance of Aerosols in the Human Respiratory Tract, Salzburg 18-20 September, 1986 (Edited by W. Hofman) Facultas Universitätsverlag pp. 118-122.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Università degli Studi di Milano
Via Festa del Perdono, 7
I - 20122 Milano

Contract no.: BI6-D-177-I

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.: 1

Radiation carcinogenesis in vitro

Head(s) of project:

Lucia Tallone Lombardi
c/o Dipartimento di Fisica dell'Università degli Studi di Milano
via Celoria 16 - 20133 MILANO

Scientific staff:

D. Bettega, P. Calzolari, A. Ghidoni, E. Rimoldi
V. Tiranti, M. Princivalli

I. Objectives of the project:

Determination of the dose effect relationship for cell transformation in C3H10T1/2 exposed to 5.48 MeV alpha particles in the low dose range between (0.01-1)Gy at two dose rate, 5 10^{-3} and 1 Gy/min. Study of dose protraction effects and repair process.

II. Objectives for the reporting period:

To complete the study of the effect of the initial cell density on transformation frequency in a large cell density interval at high and low dose values. To study the growth kinetics of irradiated populations of C3H10T1/2 with the aim of highlighting the parameters of importance in the focal assays. To improve the equipment and the set-up to be used for cell exposure to low dose rates of 5.48 MeV alpha particles;

III. Progress achieved:

1) Effect of cell density. The study of the effect of cell density on transformation frequency in C3H10T1/2 exposed to 200 KVp X-rays has been completed.

Cells in the exponential growth phase were exposed to 220 KV X-rays at dose values of 7 and 0.5 Gy, survival level of 0.09 and 0.99. The cells were plated in 9 cm diameter petri dishes in appropriate number so that the resulting number of viable cells was between 25 and 2400/dish. Samples were treated following the standard technique for tranformation assays. Type II and III foci, following the criteria already established were scored as transformed cells. Surviving fractions were determined in parallel.

Data at 7 Gy, already reported, have shown that the transformation frequency for surviving cell (TRV) is constant in the density interval between 50 and 200 cell/dish, then drops by a factor of 5 between 200 and 700. Above 700 it again remains constant within the range measured up to 2500 cell/dish. This behaviour is in agreement with data reported in the literature with low LET radiations for high dose values (greater that 2.5 Gy). No data on low dose have been reported. We have done a systematic determination at 0.5 Gy in the same experimental condition. At 0.5 the frequency is again constant between 50 and 200, then drops rapidly until 600 cell/dish decreasing by a factor of 10. Above 600 another plateau seems to be present. One can therefore conclude that there are two regions in which it could be convenient to work the first in the range between 50 and 200 cells/dish and the second at density values higher than 600. Moreover ratio between higher and low doses values in the two regions are quite different.

2 Growth kinetics of C3H10T1/2,Growth curves of population irradiated with dose up to 7 Gy of low LET radiation were determined by counting the cells in a Coulter Counter in the time interval between 90 and 900 h, and the number and dimension of each colony in the interval 5-100 h, at the aid of a microscope.

At the present we are carrying out the analysis of these data. Preliminary results show that a) in the first 200 h after irradiation the doubling time is an increasing function of the dose; b) a large part of the non surviving cells is present as single viable cell in the interval studied up to 190 h; c) the colony size is a decreasing function of the dose: ratio between mean number of cell/colony of the control and at 7 Gy resulted to be greater than 2.

IV. Objectives for the next reporting period:

To determine transformation frequency in C3H10T1/2 exposed to 5.48 MeV alpha form ^{238}Pu in a dose range of less than 1 Gy at two dose rates $5 \cdot 10^{-3}$ and 1 Gy/min.
To complete the analysis of the data on growth of the C3H10T1/2 exposed to various doses of X-rays.

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

VI. Publications:

Split-dose effects in C3H10T1/2 exposed to low LET radiations. D. Bettega, P. Calzolari and L. Tallone Lombardi. 20th annual meeting of the European Society for Radiation Biology. Pisa, sept. 15-19, 1986 in Int.J.Radiat.Biol. 51,933, (1987)

Radiocarcinogenesis: results from in vitro experiments. D. Bettega, P. Calzolari, and L. Tallone Lombardi. Il Nuovo Cimento, september (1987).

Cell Density Effect on Transformation. D. Bettega, P. Calzolari, and L. Tallone Lombardi. 8th International Congress of Radiation Research. Edinburgh, 19-24 July 1987.

Effects of split-dose irradiation on survival and oncogenic transformation induced by 31 MeV protons in C3H10T1/2 cells. D. Bettega, P. Calzolari and L. Tallone Lombardi. Int. J. Radiat. Biol., 52 ,761-765, 1987

Effetto della densità cellulare sulla frequenza di trasformazione in sistemi in vitro. D. Bettega, P. Calzolari, e L. Tallone Lombardi. IV Convegno Società Italiana per le ricerche sulle radiazioni; 15-16 settembre 1987. C.R.E.A., S. Teresa, ENEA, Lerici, (SP).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Kernforschungszentrum
Karlsruhe GmbH
Postfach 3640
D - 7500 Karlsruhe 1

Contract no.: BI6-D-091-D

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	Dr. F.Schuler
Dr. F.Planas-Bohne	L. Yule
Dr. W.G.Thies	P. Gaskin

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:

The objectives for 1987 were as follows:

1. Conclusion of the studies of the role of transferrin in the cellular uptake of plutonium.
2. Further development of the liver cell spheroid as a model for in vitro studies of the deposition of actinides in liver.
3. Continuation of the studies of the speciation and fractionation of actinides in the simulated gastro-intestinal tract model.
4. In vitro and in vivo studies of the binding of plutonium, neptunium and protactinium to serum proteins and to tissue components and their mobilisation by chelating agents.

III. Progress achieved:

1. The Role of Transferrin in the Cellular uptake of Plutonium

The investigations of the potential role of the metal-transferrin complex-transferrin receptor system in the uptake of plutonium have been continued in both HeLa cells and in rat liver cell spheroids. In both systems iron does appear to be taken up into cells via this mechanism, but although plutonium is bound to the cell membranes in quite large amounts, there is very little transfer of plutonium to the cell interior when transferrin is present in the medium. It is not yet clear whether the Pu-transferrin complex binds, irreversibly, to the transferrin receptor, or whether the membrane-binding involves a site other than the transferrin receptor.

2. Studies with Liver Cell Spheroids

In continuation of our search for in vitro systems which may be used to model organs in vitro, the liver cell spheroid system has been further developed. Spheroids have been prepared from pure rat hepatocytes and from mixtures of hepatocytes, endothelial cells and Kupffer cells and compared in relation to the uptake of Plutonium and iron and of the retention of important liver enzymes, GOT, GPT and Cytochrome P450. Biochemical functions such as collagen synthesis have also been examined. All the results suggest that the liver cell spheroid is a very useful system for biochemical and toxicological studies, and does possess some advantages over monolayer, or single cell suspension cultures. However, its further applicability to speciation studies appears to be limited.

3. Speciation and Fractionation of Actinides in a Simulated Gastro-intestinal Tract Model.

The in vitro studies with a simple gastro-intestinal model system were continued using Pu-238, Np-239 and Pa-233. The results confirmed the previous observations that the presence of diet derived complexing agents plays the most important role in determining the chemical fractionation of these actinides near the absorptive surfaces in the duodenum. The model system was refined to yield more realistic simulation of conditions in the duodenum. Further analytical methods, ion-exchange chromatography, electrophoresis, have been introduced in an attempt to obtain more information on the species present. These studies indicate that for Pu-238 about 90% of the nuclide is present as negatively changed species and about 3% as positively charged complexes, neutral,

absorbable complexes represent only a very small fraction of the total plutonium in the duodenum. New methods need now to be developed to gain more information on the exact nature of the actinide species present on or near the absorption sites in the duodenum.

4. In vitro and in vivo Studies of the Binding of Actinides and related Elements to Transferrin and other Proteins.

a) The Binding of Protactinium to Transferrin. The observation that in vivo Pa was bound to the serum protein transferrin, has been confirmed by in vitro studies with pure human transferrin and with rat serum.

b. In vitro studies of the binding of iron, plutonium and hafnium to transferrin have been carried out using ultra-violet difference spectroscopy. The results show that stoichiometrically and mechanistically the binding of Fe(III), Pu(IV) and Hf(IV) are similar. However, in vitro and in vivo studies show that the metabolic behaviour of these three elements is not identical and these differences may reflect important changes in protein confirmation resulting from the binding of the specific metal. Such conformational changes may be of importance in relation to the formation, and or dissociation of metal-transferrin - transferrin receptor complexes on cell membranes. Further information of the mechanisms of binding of hafnium is being obtained by the time-dependent perturbed angular correlation of gamma rays using Hf-181.

c) Studies of the subcellular distribution of Pa-233 in rat liver in vivo have been continued, the results show that an entering the liver cell cytosolic Pa is bound rapidly to a protein of molecular mass ca 200 kDa and also to a protein of ca 80 kDa, probably transferrin. Within hours the metal migrates from these proteins to bind to a protein of >400 kDa, which appears to be ferritin. The ultimate storage site appears to be the lysosomes with much lesser amounts of the Pa bound to another, so far unidentified organelle.

d. Studies of the ability of various chelating agents, including several hydroxypyridone derivatives, and LICAM(C) to mobilise actinides in vivo have been continued. A particularly interesting observation has been the mobilisation of Np-239 from rat liver and skeleton by DTPA, Desferrioxamine or LICAM(C). In collaboration with EULEP laboratories a new pure sample of LICAM(C) was prepared and both in vivo and in vitro tests were begun.

IV Objectives for the next reporting period.

1. Continuation of the studies of the speciation and fractionation of actinides in the improved simulated gastro-intestinal tract model.
2. Spectroscopic and chromatographic studies of the binding of actinide and selected 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 gastro-intestinal tract and in the human food chain.

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

National Radiological Protection Board
Chilton, Oxfordshire, UK

IPSN Fontenay aux Rose, Bruyeres le Chatel, France

Department of Applied Chemistry, University of Wales, Cardiff, UK.

Radiobiology Dept. SCK/CEN Mol. Belgium

European Late Effects of Radiation Project (EULEP)

Department of Haematology, Royal Free Hospital, London, UK.

VI Publications:

Schuler,F., Taylor,D.M.. The subcellular distribution of Pu-239 in primary cultures of rat hepatocytes. Radiat. Res. 110, 362-371. 1987.

Taylor,D.M., Farrow,L.C.. Identification of Transferrin as the main Binding Site for Protactinium in Rat Blood Serum. Nucl. Med. Biol. 14, 27-31, 1987.

Taylor,D.M., Seidel,A., Planas-Bohne,F., Schuppler,U., Neu-Müller,M., Wirth,R.E.. Biochemical Studies of the Interaction of Plutonium, Neptunium and Protactinium with Blood and Liver Cell Proteins. Inorg. Chim. Acta 140, 361-363, 1987.

Appel,H., Duffield,J.R., Taylor,D.M., Then,G.M., Thies,W.-G. TDPAC Studies of Hf-181 labelled transferrin: Comparison between human and rat serum transferrin. Hyperfine Interactions 35, 957-960, 1987.

Duffield,J.R., Taylor,D.M.. A Spectroscopie study on the binding of plutonium (IV) and its chemical analogues to transferrin. Inorg. Chim. Acta 140. 365-367, 1987.

Volf,V., Wirth,R.. Effective chelation therapy after incorporation of neptunium-239 in rats. Int. J. Radiat. Biol. 50, 955-959, 1986.

Schuler,F., Csovcsics,C., Taylor,D.M.. Differences in the uptake of transferrin bound Pu-239 and Fe-59 in the multicellular spheroids of hepatocytes from adult male rats. Int.J.Radiat.Res. 52, 883-892, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Trinity College
IRL - Dublin 2

Contract no.: BI6-D-184-IRL

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.: 1. A study of the transformation of primary human cell cultures by radiation and radiation in combination with environmental chemicals.

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 investigate the effect of B-propriolactone and Benzo-(a)pyrene in combination with radiation on oesophageal mucosa with particular emphasis on time of exposure.

(2) To study the effect of N-Nitrosodiethanolamine ± radiation on oesophageal mucosa with particular emphasis on the detection of changes suggesting transformation.

(3) To look at the effect of radiation and aniline dyes on bladder urothelium and to study the effect of radiation + DMBA or DMH on breast and colonic explants respectively.

(4) To continue to study LDH isoenzyme and GST in treated cultures.

(5) To continue attempts to quantify the changes in growth and morphology using cytophotometric, ultrastructural and autoradiographic analysis.

III Progress achieved:

METHODOLOGY: The culture methodology is being developed in parallel with Contract No. B16-D-092-IRL and is detailed in that report.

Environmental carcinogens are being selected on the grounds that (1) they cause cancer in humans in the tissues being cultured, (2) there is a realistic possibility that human populations could be exposed to both radiation and the chemical. Radiation is being used as both an initiator and a promotor of carcinogenesis and cells are exposed at various times in relation to the time of exposure to the chemical carcinogen. The C3H $10T\frac{1}{2}$ transformation system is being used to determine suitable ranges of carcinogen and radiation doses.

Epidemiological data is being obtained from European Cancer Registries and 'hot spots' for cancers in the organs of interest in this project are now being assembled.

RESULTS: (1) The escape from growth control induced by radiation in combination with Benzo(a)pyrene or β-propriolactone has been confirmed in oesophageal mucosa, but no increased growth occurred in bladder urothelium.

(2) Nitrosamines which showed a marked synergism when added in combination with radiation to cultures of oesophageal mucosa did similarly stimulate bladder urothelium (see Table 1a). A series of experiments set up to follow up this effect using urothelium obtained from the ureters of normal kidney transplant donors revealed that, in addition to increased growth of cells, changes in morphology of cells (seen with light and electron microscope) and changes in the LDH isoenzyme profile of the exposed cells could be detected.

(3) Increased proliferation of bladder urothelium was also detected with certain combinations of aniline dyes and radiation. Aniline dyes are known to cause bladder cancer in exposed humans. Dimethylhydrazine, a bile acid degradation product suspected of being involved in the etiology of colon cancer, similarly caused increased proliferation in colonic epithelial cultures, when cells were subsequently exposed to radiation (Table 1b). The mechanism of the radiation effect is unknown but it is likely to be acting as a promotor in these studies. Attempts to study radiation/carcinogen interaction in breast tissue were hampered by the lack of availability of normal surgical samples of human breast tissue in this country.

(4) The problems experienced with LDH and GST assays in project B16-D-092-IRL also apply in this project but are slightly less because of the increased growth occurring following combined carcinogen/radiation exposure. However, it is considered essential to develop a cytochemical method for looking at these substances. This is particularly important because such assay methods allow two or more antigens to be detected in the same cell, which means LDH profiles in the proliferating fraction versus the quiescent fraction could be studied.

(5) Autoradiographic analysis has been used to quantify the increased proliferation following exposure of explant cultures to radiation and carcinogens. The use of the new proliferating cell antigen K 167 which detects all cells which are cycling is also being used. Cytophotometry of normal and tumour explants to establish ploidy ranges which could be expected following transformation have also commenced and are now being applied to carcinogen treated normal cultures. Small changes in ploidy have been detected and significant increases in nuclear : cytoplasmic

volume can be detected in distant progeny of irradiated cells.

(6) Ultrastructural studies of progeny of cells exposed to combinations of radiation and carcinogen (nitrosamine) are continuing. Difficulty is being experienced in sorting out changes due to toxic effects from those which might be suggestive of early carcinogenic change. Particularly obvious changes are those occurring in the nuclear membrane and in the mitochondrial membrane. These are apparent in all cells in the explant outgrowth which were examined and may, therefore, be heritable changes since treatment of the explant occurred two days after plating and before more than a few hundred cells had emerged from the parent tissue.

TABLE 1a THE EFFECT OF N-ETHYL NITROSAMINE ± 5 GY ON THE EXTENT OF CELLULAR OUTGROWTH DETECTED AFTER FOUR WEEKS IN PRIMARY EXPLANT CULTURES OF HUMAN UROTHELIUM

Treatment	% Outgrowth	Stimulation by Nitrosamine
Control	100	-
Control + 5 Gy	22.7	-
0.05 µg/ml Nitrosamine	163	1.63
0.05 µg/ml Nitrosamine + 5 Gy	109	4.8

TABLE 1b THE EFFECT OF DIMETHYLHYDRAZINE (DMH) ± 5 GY ON THE EXTENT OF CELLULAR OUTGROWTH DETECTED AFTER FOUR WEEKS IN PRIMARY EXPLANT CULTURES OF HUMAN COLONIC MUCOSA

Treatment	% Outgrowth	Stimulation by DMH
Control	100	-
Control + 5 Gy	52	-
0.1 mM DMH	48	0.48
0.1 mM DMH + 5 Gy	233	4.48

DISCUSSION: The technique used is consistently showing increased growth following treatment of target organ explants with chemical carcinogens. Radiation in general augments the effect of the chemicals and, in the case of DMH and nitrosamine, very strongly augments the effect. It is interesting that these two compounds are dietary factors. The major experiments remaining to be done on this aspect of the work concern analysis of the increased proliferation to determine whether it involves a small increase in growth rate in all cells in the explant or a large increase in small groups of cells.

The other main area of the project requiring intensive work is the development of methods for microanalysis of oncogene activation, isoenzyme shifts, etc. We are convinced that immunocytochemical techniques will allow qualitative demonstration of changes. Quantitative analysis of changes will be more difficult to demonstrate.

IV Objectives for the next 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 notrosamines, 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.

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

NONE

VI Publications.

Listed with Contract No. B16-D-092-IRL

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-094-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. 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:

Greet Schoeters

Scientific staff:

O.L.J. Vanderborght
G. Schoeters
S. Van Puymbroeck

I. Objectives of the project:

Research sponsored by the former CEC contract BIO-D-381-B showed that 50% decrease of ^{226}Ra content did not alter substantially the osteosarcoma incidence in mice. Why is a substantial decrease of body-burden by decorporation treatments (such as was obtained with ^{226}Ra in mice) not reflected in a decrease of bone cancers ? Is this lack of long-term protection linked to the characteristics of the radioisotope ? Does it hold also for other high risk isotopes such as ^{241}Am ?

II. Objectives for the reporting period:

1. Soft tissue histology of suspicious bone fragments diagnosed on post-mortem radiographs from male C57Bl mice derived from a large scale survival experiment which includes 860 mice. The experiment evaluates on one hand the long-term effect of chronic treatment with Zn-DTPA injections during 8 successive weeks and starting 4 days after contamination of mice which received 10 and 1.5 kBq ^{241}Am per mouse. On the other hand the efficiency for osteosarcoma induction after ^{241}Am injection is compared with the effects of ^{226}Ra on the same mouse strain.
2. Female Balb/c mice, also of SCK/CEN breeding, were evaluated on their sensitivity for developing late radiation effects after injection of 2.1 kBq ^{241}Am citrate. The aim is to develop a model for osteosarcoma induction with a higher yield of osteosarcomas after alpha-radiation than is found in C57Bl mice (SCK-inbred).
3. Investigation of translocation of ^{226}Ra after 6 months of Na-alginate treatment in vertebrae and femurs of mice which were injected with 24 kBq $^{226}RaCl_2$.

III. Progress achieved:

Methodology

1. Post-mortem radiographs were examined and suspicious bone fragments were decalcified in EDTA for 2 months. The tissues were embedded in paraffine, sectioned and stained with hematoxylin-eosine. The histopathology was observed.
2. All the ^{241}Am injected female Balb/c mice had died and radiographs were taken and examined.
3. The ^{226}Ra content was measured of some of the dissected femurs and vertebrae via gamma-spectrometry. Remaining vertebrae and femurs were transported to NRPB (Harwell, U.K.) to perform autoradiographs using CR39.

Results

1. The yield of malignant bone tumors was very low in the male C57Bl mice which were injected with ^{241}Am citrate. The maximum yield was 6.9% (with 95% confidence limits of 2.9% and 13.8%) and was obtained after injection of 1.5 kBq ^{241}Am per mouse resulting in an average cumulative skeletal dose at death of 4.7 Gy. Injection with an ^{241}Am dose which was 3 times higher resulted in no bone tumors in a group of 106 mice. A dose of ^{241}Am which was 3 times lower resulted in 4.1% bone tumors (1.1-10.2%). ^{226}Ra injected in a similar group of mice included in the same experiment resulted in 11.2% bone tumors (5.3-20.3%). 156 control C57Bl mice did not develop any bone tumor. C57Bl mice treated with Zn-DTPA after injection with 1.5 kBq ^{241}Am per mouse showed 3.9 (1.1-9.7)% bone tumors in a group of 103 mice. This was not significantly different from the 6.9% in non-treated mice injected with the same dose of ^{241}Am. The mean induction time of osteosarcomas in non-treated mice was 497 + 136 days after contamination, in Zn-DTPA treated mice it was 591 $\pm$ 160 days.
2. From the radiographs we did a preliminary estimation of obvious bone deformations, however, further histopathology needs to be done in order to diagnose which of the lesions are bone tumors. 48% of the mice showed deformations of their skeleton.
3. A 6 months lasting alginate treatment (5% of daily diet) starting 4 days after injection of $^{226}RaCl_2$ (24 kBq/mouse) in adult male C57Bl mice reduced significantly the ^{226}Ra burden in the vertebrae by 30%, while in the femora the reduction was not statistically significant.

Discussion

The doses of ^{241}Am we used in our large scale ^{241}Am experiment in male C57Bl mice ranged between 0.5 kBq and 33 kBq ^{241}Am per mouse but the yield of bone tumors only went up till 7% at 1.5 kBq ^{241}Am injected per mouse. At doses of ^{241}Am, which induced 27% of bone cancers in male CBA mice (Nilsson and Broome-Karlsson, 1976, Acta Radiologica Phys. Biol. 15, 49-70), we did not detect any bone tumor. Our mice survived this dose with only 173 $\pm$ 14 days while in the CBA mice the mean induction time for the bone tumors at this dose is 352 $\pm$ 9.1 days. The C57Bl mice we used were thus very sensitive to ^{241}Am and this resulted in a dramatic decrease in survival time. ^{241}Am killed the mice before tumors could appear. The C57Bl mice did survive the injected dose of ^{226}Ra (24 kBq) longer. This dose resulted in 11% (5.3-20.3)% of bone tumors. This frequency did not differ significantly from the incidence which we observed in a previous large scale survival experiment in which various doses of ^{226}Ra were used.

No increase in liver tumors or haemopoietic malignancies was observed in the ^{241}Am injected mice.
In two dose groups half of the mice were treated with Zn-DTPA. The beneficial effect of Zn-DTPA treatment after 10 kBq ^{241}Am per mouse was a significant increase in life span. At 1.5 kBq ^{241}Am per mouse Zn-DTPA treated mice also lived significantly longer and the bone tumor yield was 3.9 (1.1-9.6)% with a mean induction time of 591 ± 160 days, while in the non-treated mice the induction time for 6.9 (2.9-13.8)% of bone tumours was 497 ± 136 days. These differences are not statistically significant at a significance level of 5%.

The female Balb/c mice seem more sensitive for bone malignancies after ^{241}Am contamination and have a low spontaneous incidence of bone tumors. They can be used as an alternative model for the study of effects of decorporation therapy. The aim is then to obtain statistically significant results with less animals than if using C57Bl mice.

IV. Objectives for the next reporting period:

1. Comparison of the survival data and pathology data of the C57Bl mice contaminated with ^{241}Am and with ^{226}Ra (previous experiment).

2. Pathology of Balb/c mice contaminated with 2.1 kBq ^{241}Am. To evaluate further the suitability of this mouse model for the study of bone tumors after decorporation therapy, the experiment will be expanded with 250 mice, male mice which will receive also 2.1 kBq ^{241}Am and females which will receive either 1 or 4 kBq ^{241}Am per mouse, control animals will also be included.
3. Interpretation of autoradiographs prepared at NRPB (Harwell, U.K.).

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. Maisin)
- Inst. für Biologie, Gesellschaft für Strahlen- und Umweltforschung, mbH, D-8042 Neuherberg, F.R.G (Dr A. Luz)
- National Radiation Protection Board, Chilton, Didcot, Oxfordshire OX11 ORQ, (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. Submitted to Health Physics, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
Rijksuniversiteit Leiden
Wassenaarseweg 72, P.O. Box 9503
NL - 2333 AL 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:

We have reported last year that considerable difficulties were encountered in our own and many other laboratories with the expression of genes that were introduced into bone marrow stem cells with the use of retroviral vectors. Although the proviral DNAs were readily integrated into the spleen colonies arising after reconstitution of lethally irradiated mice with the infected bone marrow cells, the inserted gene was not expressed or expressed at very low levels. It was decided, therefore, to discontinue this approach and try to use transgenic mice harboring an activated oncogene. These activated oncogenes should also be expressed in blood cells. Since our main goal was to examine which type of radiation or chemical agent can activate a genetic determinant that can complement a mutated ras gene attempts have been made (as part of a different project) to isolate transgenic mice harboring an activated N-ras gene with a weakly transforming potential.

III. Progress achieved:

1. Methodology

Transgenic mice were generated according to standard procedures in collaboration with Dr. E. Zwarthof (Rotterdam). The ras gene used was the human N-ras with an activating mutation in codon 13, regulated by the H-2 promoter.

2,3. Results and discussion

Despite repeated attempts so far no transgenic mice are available harboring an activated N-ras gene. Although one mouse was isolated containing the ras gene integrated in its genome, it has not been possible to transmit the introduced gene to the offspring. Since transgenic ras-containing mice could not be isolated in other laboratories either this part of the project was discontinued for the time being. In the mean time, transgenic mice containing the c-myc or the pim oncogene have been established by Dr. Berns (Amsterdam). As soon as sufficient mice are available, the project will be continued with bone marrow or fibroblastic cells from these animals (collaboration with Dr. Berns).

For more details of the experimental procedures, see project 2.

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)]:

Dr. A. Berns (Netherlands Cancer Institute, Amsterdam)

The Radiobiological Institute TNO, Rijswijk (Prof.D.W. van Bekkum)

The Department of Radiation Genetics and Environmental Mutagenesis, University of Leiden (Prof.P.H.M. Lohman, and Prof.G.R. Mohn

VI. Publications:

Title of the project no.:

Induction of oncogenic transformation in vitro
Contract nr. B16-D-185-NI

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. B16-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:

Introduction of activated ras or myc genes into primary cultures of rat embryo fibroblasts, or established rodent cell lines. Studies of the effect of these genes on cellular behaviour, in the absence of additional treatment.

III. Progress achieved:

1. Methodology

Primary cultures of rat embryo fibroblasts were transfected with the activated H-ras gene or with various constructs of the c-myc gene. The transfections were carried out in the presence or absence of the neomycin resistance gene as a dominant selectable marker.

2,3. Results and Discussion

Transformed foci appeared in all transfected cultures, which were hardly distinguishable from the foci arising after transfection with a combination of activated ras and myc. Treatment with TPA did not have additional effect. From these preliminary results it was concluded that the rat embryo fibroblasts system used was not suitable for our purpose, since a single oncogene already could cause full morphological transformation in a too high frequency. Even if semi-transformed colonies could be obtained by G418 selection, the spontaneous rate of appearance of secondary events would be too high to obtain meaningful results. In the meantime, the established mouse 10T½ and the rat 3Y1 cell line were transfected with the activated ras gene, in the presence of a dominant selectable marker. The cells were tested for the presence of the mutated ras gene and only 10T½ cells could be isolated with the activated ras gene. The 10T½ cells will be tested for their ability to grow in semi-solid medium and for tumorigenicity. If the cells are negative for these properties they will be irradiated with either X-rays or UV-light, and the induction of full morphological transformation will be scored. In addition, the cells will be treated with the following chemical agents as control: ENU, Mitomycin-C (or Cis-Pt) and acrylamide. In this way, we hope to obtain information as to which of the treatments tested can induce the carcinogenic event(s) that can complement an activated ras gene to obtain full oncogenic transformation.

Depending on the outcome of these experiments, similar studies can be carried out with cells harboring an activated myc oncogene. The two sources of myc-containing cells available are: established cell lines with a transfected c-myc gene, or primary cultures obtained from transgenic myc mice (see project 1).

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)]:

The department of Radiation Genetics and Environmental Mutagenesis, University of Leiden (Prof. P.H.M. Lohman, Prof. G. R. Mohn)

The Radiobiological Institute TNO, Rijswijk (Prof. D.W. van Bekkum)

Dr. A. Berns, The Netherlands Cancer Institute, Amsterdam

VI. Publications:

J.L.M. van der Lubbe, Effects of ultraviolet irradiation on mutagenesis and induction of latent viruses in mammalian cells. Thesis, Leiden 1987.

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. accepted 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 1987 in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-D-097-UK

Welsh National School of Medicine
Heath Park
GB - Cardiff CF4 4XN

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 set up the conditions for radiation; to check the calibration of the radiation equipment; to irradiate groups of animals; to confirm that the protocol used will give adequate numbers of tumours so that the effect of cessation of the TSH stimulation can then be studied.

III. Progress achieved:

Method (A): One hundred and fifty two ten week old male Wistar rats weighing 297 ± 28 gms were divided into four groups of equal size: A, B, C and D. The thyroid of each animal was exposed to various levels of X-rays from an external caesium source, i.e. 0, 1, 10 and 100 rads of X-rays to the respective groups. Seven days later a goitrogen (0.5% sodium perchlorate + 0.5% sucrose (to mask the bitter taste of the perchlorate) + 0.05% 3-amino, 1, 2, 4-triazole) was added to their drinking water which was available to them 'ad libitum' . From this point in time the goitrogen was present in their drinking water throughout the experiment. The effectiveness of this goitrogenic solution had been previously analysed and found to reduce the uptake of radioiodine to less than 2% of the uptake of the controls.

I	I----------I----------I		
+0,1,10	7days	8months	14months
or 100rads	+ goitrogen		

Half the animals from each group were sacrificed 8 months after the start of the experiment, the other half being sacrificed 6 months later at 14 months.

The thyroids were resected on the trachea, one lobe being fixed overnight in 10% formol saline, the other being snap frozen in liquid nitrogen for p53 analysis. The fixed lobes were then dissected off the trachea and weighed before being embedded in wax. Sections of 4µ were taken at 100µ levels throughout the lobe. These sections were stained with haemotoxylin and eosin and examined histologically for the number, size and types of lesions present.

Method (B) : One hundred and thirty-five seven week old male Wistar rats, weighing 139 ± 23 gms were divided into 14 groups (see Plan).

On day 0 every animal in each group, except K, received 10µCi of 131-I. Goitrogen [1% sodium perchlorate + 1% sucrose (to mask the bitterness of the perchlorate)] was added to the drinking water which the animals were free to drink 'ad libitum' twenty-fours hours later, on day one. The goitrogenic regimen was initiated and adhered to for the course of the experiment. A pilot study, consisting of groups A, B and C (5 animals per group) had suggested that there were fewer tumours per animal in those animals that had been deprived of the goitrogen for 4 days between days 47 and 51, i.e. group B showed fewer tumours compared with group A. In order to confirm this observation the experiment has been

repeated and extended to include the groups shown in Table 2.

The animals were sacrificed, the thyroids resected on the tracheae (which was bissected in order to separate the lobes of the thyroid) and fixed overnight in 10% formol saline. The thyroid lobes were dissected off the tracheae, embedded in wax and 4μ sections at 100μ levels were taken. The sections were stained in haemotoxylin and eosin and examined histologically.

Results (A): Results of histological examination at 8 months; those from the 14 month group will be available in the near future.

Group	A (0 rads)	B (1 rads)	C (10 rads)	D (100 rads)
No animals	19	19	19	19
Mean tumour no. per lobe	1.2	0.5	0.5	1.4
std. dev.	1.1	1.1	0.6	1.5
Mean lesion no. per lobe	2.4	2.3	1.7	2.9
std. dev.	2.5	4.7	2.6	2.9

where: tumour no. = number of tumourous lesions

lesion no. = number of tumourous + non-tumourous lesions

std. dev. = standard deviation

Conclusion: Statistical analysis (Student's t-test) shows that there is no significant difference between the groups with respect to both tumour number and lesion number.

Results (B): The results of the groups thus far examined are given below:

Table 1

Group	A	B	C	C'	E'	F'	K
Animal no. per group	5	5	10	10	10	10	5
Mean tumour no.	4.9	2.6	0	0.1	0.65	2.3	0
std dev.	3.5	1.8	0	0.31	1.04	1.5	0

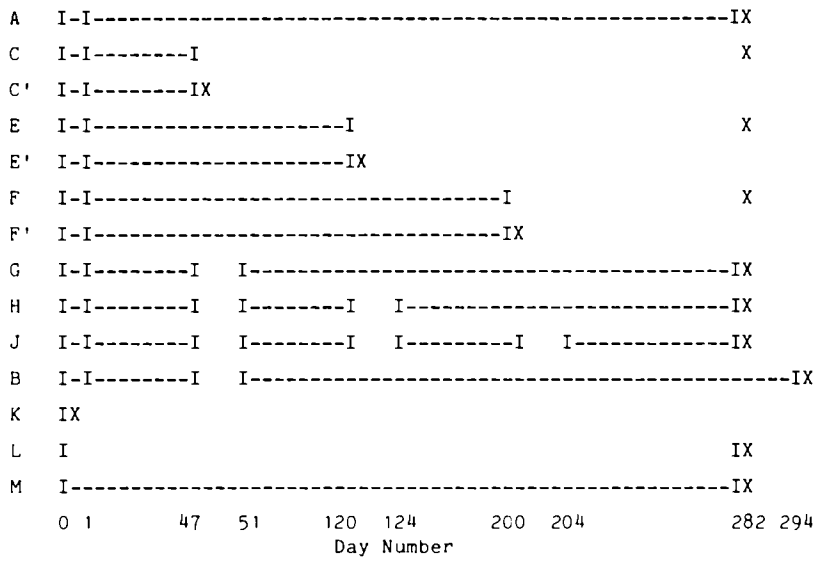
Table 2: Ratio and percentage of animals per group with tumours

Group	Ratio	Percentage
A	5/5	100
B	4/5	80
C	0/10	0
C'	2/10	20
E'	6/10	60
F'	10/10	100
K	0/10	0

C nclusions

1. The mean number of tumours per lobe increases with time provided the goitrogenic stimulus remains
2. The percentage of animals per group with tumours also increases with time provided the goitrogenic stimulus remains
3. Statistical analysis (Student's t-test) of tumour number per lobe in groups A and B suggest tnat there is >90% chance that there is a significant difference between these groups.

Plan



--- = presence of goitrogen in the drinking water
X = time at which animals were sacrificed

Discussion: These results are essentially interim results, during the next few months both main projects will reach a stage at which important results will become available; firstly to provide accurate data on the carcinogenicity of low level irradiation in the goitrogen induced thyroid tumour level, and secondly to provide information on the effects of temporary or permanent interruption of the goitrogenic stimulus on the relationship between rad dose and carcinogenicity.

IV. Objectives for the next reporting period:

To complete and analyse the first phase of the experiment designed to show (a) the relationship between low dose external radiation of the thyroid and the number of tumours induced in the goitrogen model, and (b) to study the effect of temporary or permanent interruption of the growth stimulus on carcinogenesis.

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

None

VI. Publications:

None

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

TNO Institute
for Experimental Gerontology
Juliana van Stolberglaan 148
Postbus 297
NL - 2501 BD 's-Gravenhage

Contract no.: BI6-D-212-NL

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
Dr. S.K. Durham/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:

To select multiple cases of various categories of benign and malignant rat mammary tumours.
To prepare nuclear suspensions from formalin fixed and paraffin embedded rat mammary tumour material, with optimal nuclear morphology, allowing application of morphometric methodology in addition to DNA flow cytometric analysis.
To investigate whether nuclei from one of the parenchymatous tissues from the same animal which was processed under identical conditions as the mammary tumour tissue could be used as an internal standard for the Go/G1 peak of normal tissue.

III. Progress achieved:

A series of rat mammary tumours was selected from our files exhibiting all gradations from histologically benign to malignant as represented in the Table.

Table

Benign and malignant rat mammary tumors selected for flowcytometric and morphometric analysis

	Diagnosis	Number
Benign	tubular adenoma	20
	papillary cystadenoma	20
	fibroadenoma	20
Malignant		
	noninvasive	
	tubulopapillary carcinoma	20
	cribriform-comedo carcinoma	20
	invasive	
	tubulopapillary carcinoma	7
	cribriform-comedo carcinoma	19
	carcinoma in fibroadenoma	20
	metastasizing carcinoma	7

Fifty µm sections were prepared from paraffin blocks of selected benign and malignant rat mammary tumours, cleared of paraffin by two changes of xylene of 10' each and stepwise rehydrated in a series of alcohol 100%, 96%, 70% and 50% to distilled water. Each step took 10 minutes at room temperature and fluids were changed twice. The rehydrated tissue sections were washed twice with phosphate buffered saline (PBS) and subsequently subjected to enzymatic digestion to prepare a suspension of free nuclei.

In order to develop a method for the preparation of single nuclei suspensions of rat tumour tissue with optimal morphology of the nuclei allowing also morphometric analysis, several enzymes were tested, i.e. pepsin 0.5% (pH 1.5 or 1.9), trypsin 0.5% pH 7.4, pronase 0.05% and several proteases. Of these digestive enzyme preparations, protease 0.05% (protease XXIV Sigma P-8038) gave the best results.

The tissue sections were incubated for 30 min in 3 ml 0.05% protease XXIV at pH 7.3 at 37°C. The digestion was stopped by adding 10 ml cold PBS. After vortexing the suspension was filtered through a 30 µm mesh nylon gauze, centrifuged, washed with PBS, resuspended and passed 2 times through a 27 gauge needle. The final volume was adjusted to about 10^6 nuclei/ml. A cytospin preparation was made to check for nuclear fragments, clumping of nuclei and optimal morphological appearance.

The nuclei were stained with propidiumiodide 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 (FACS II, Becton Dickinson, Sunnyvale, CA) equipped with an argon laser operating at 488 nm (0.5 W).

Interpretation of some of the resulting histograms appeared difficult. For instance it was impossible to decide whether a rather broad Go/G1 peak [coefficient of variation (cv) > 10%] was due to an artifact of the formalin fixation and embedding procedure, or was a characteristic of the specific tumour nuclei. We therefore decided that it would be necessary to have an internal standard consisting of nuclei derived from one of the normal parenchymatous tissues of the same animal which was processed (e.g. formalin fixation, paraffin embedding etc) simultaneously and under identical conditions. We therefore investigated whether nuclei prepared from formalin fixed and paraffin embedded liver or renal tissue could be used as an internal standard for calibrating the apparatus for the peak channel of the Go/G1 peak and for measuring the cv value inherent to the processing conditions of necropsy material of a specific animal. It appeared that renal tissue proved to be superior to liver tissue, as cytoplasmic remnants around the liver nuclei were difficult to remove with the enzyme solutions tested and clumping of nuclei was frequent. A cv of less than 8% was found for most suspensions of nuclei prepared from renal tissue.

IV. Objectives for the next 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 standardised 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.

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

1987

Contractor. Contract no : BI6-D-099-D

European Late Effects
Project Group - EULEP
Rue Charles Lemaire, 1
B - 1160 Bruxelles

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

Dr. J.R. Maisin
UCL - Faculté de Médecine
Unité de Radiobiol. et de Radiothér
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
Département de Biologie
C.E.N./S.C.K.
B-2400 MOL (Belgium)

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

The results of the 4th and 5th dosimetry intercomparisons for whole body irradiation of mice were unsatisfactory for one of the participating institutes. For the central position inside a polymethylmethacrylate (pmma) phantom the dose quoted by this institute relative to the dose determined by the reference institute was ca. 0.83. A site visit was made to resolve the discrepancies. It was concluded that the conversion factor for exposure at the centre of the pmma mouse phantom to exposure at the monitor chamber was different by ca. 12% from the factor employed previously. This is most likely related to a change in the monitor position after the third dosimetry intercomparison. A 17% discrepancy can be explained on the basis of this and other observations made.

These results and the adopted modifications were confirmed using mouse phantoms filled with TLD (see A.H.L. Aalbers and F.J.M. Bader, The fifth EULEP dosimetry intercomparison, RIVM Report no. 24800500, 1986). The results indicated good agreement for absolute dosimetry for the three exposure arrangements at this institute.

New activities being planned include a second EULEP-EBMT workshop on the physical, biological and clinical aspects of total body irradiation. The studies on animal phantoms included in the EULEP dosimetry protocol will be extended to more realistic phantoms.

Committee of Internal Radiation Dosimetry and Techniques

The Committee met on two occasions, during the Reisensburg meeting in March and in Antwerp on 28 September. On the former occasion the Committee also organised a one-day workshop on "Macrophages"; this was part-educational with critical review papers from world-renowned experts in various aspects of macrophage function, and partly provided a forum for the detailed discussion of the work on macrophages being carried out in EULEP laboratories. This very successful meeting initiated a number of new collaborative ventures between member laboratories. A part of the Antwerp meeting was devoted to a scientific discussion of some problems of beta-dosimetry. The Committee has continued to provide advice and assistance to those

task groups which come within its purview.

Work has continued on the provision of a simple data base and calculation system for the assessment of radiation dose following ingestion or inhalation of the important radionuclides likely to be encountered in a nuclear accident. A system based on a single floppy disc to be used on any IBM-compatible personal computer has been designed and is now in the final stages of editing and testing. It should contain data for about 25 radionuclides in about 20 organs or tissues for children of various ages and for adults.

Committee of Pathology

Two workshops were organised by the Committee. The first, on 10 March preceding the General Assembly, was on "Comparative pathobiology of experimental in vivo and in vitro systems". Studies on several experimental tumour models (lung, bone, colon and Harderian gland) as well as on parenchymal and nonparenchymal liver cells were presented.

The second workshop was on neuroendocrine pathology and took place on 25-26 September in Uppsala. The program included the phylogeny, normal development and proliferative lesions of the APUD system applying a variety of techniques such as immunohistochemistry, electron microscopy and in situ hybridization. Nine experts in the field of neuroendocrine pathology were invited as guest speakers. This very successful and scientifically stimulating meeting was subsidized in part by the Swedish government and industry.

Other activities of the Committee included consultation: a limited number of consultation cases were discussed during the meetings of the Committee; and the Pathology Atlas: the revised fascicle on genital tract pathology is in press and a fascicle on urothelial tumours is in preparation.

Committee of Cell and Molecular Biology

A business meeting was held on 9 March in Reisensburg, preceding the General Assembly. This was principally concerned with the final organisation of a practical course on molecular biology (see below), and with individual requests of 2 members to make working visits to laboratories in Brussels and Rijswijk, to acquire techniques of

molecular hybridization in situ and of DNA electrophoresis in denaturing gradient gels respectively.

A practical course on the Fundamentals of Molecular Biology was organised at the Department of Biology, CEN/SCK, Mol. It was attended by twelve participants, all from EULEP member or associated laboratories. The participants had the opportunity of individually purifying DNA and polyadenylated RNA from retrovirus-induced rat thymic lymphomas. They prepared radiolabelled molecular DNA probes and assessed, in restriction enzyme-digested tumour DNAs (Southern technique), the appearance of novel proviral sequences and of rearrangements in the c-myc oncogene domain. Among the polyadenylated RNAs (Northern technique), they detected the expression of viral and of nonviral, tumour-specific molecular species. Finally, they established a restriction map of a molecularly cloned proviral genome.

TASK GROUPS: Progress achieved by co-ordinated activities of the task groups was as follows:

1. Molecular approach to the study of radiation-induced osteosarcoma

The effect of FBR MSV (from radiation-induced murine osteosarcoma) on osteoblast-like cells was studied in two in vitro systems. The infection of the permanent osteoblast-like cell line MC 3T3-E1 with FBR MSV was followed by neoplastic transformation of the cells and irreversible abrogation of osteogenic differentiation during prolonged cultivation. Infection of a cartilage organ culture induced the development of a transplantable osteosarcoma-like lesion in vitro.

Bone-pathogenic murine leukemia virus (OA MuLV) enhanced osteogenic differentiation of primary osteoblast-like cells and reversibly decreased the expression of the osteoblastic phenotype of immortalized osteoblasts. Studies on migration and invasion of osteosarcoma cells and transformed fibroblasts showed a dose-dependent inhibition of chemotaxis and invasion of a reconstituted basement membrane by human leukocyte interferon and murine fibroblast interferon.

Transcriptional control and oncogenicity studies of various murine leukaemia viruses revealed distinct differences of the LTR-U3 regions of Akv, SL3-3, SL3-2, FBJ helper virus and OA MuLV. Some of these viruses were found to induce lymphomas and skeletal disorders such as osteopetrosis and osteomas.

The group met in Sandjberg on 6-8 February and in Munich on 19-20 November.

2. Molecular approach to the study of radiation-induced lymphoma

Studies have continued aimed at identifying cellular genes involved in the genesis of radiation-induced thymic lymphomas in rodents. Tests have been made for the possible expression, in RadLV-induced rat thymic lymphomas, of RNA synthesized from a cellular gene under a downstream promotion control by a newly integrated provirus. A potential candidate sequence was detected, comprising viral and adjacent cellular sequences, but the study has been complicated by the unexpected activation by RadLV of endogenous rat proviral genomes.

Kaplan's hypothesis that radiation-induction of thymomas might be directly mediated by a leukemogenic retrovirus has been tested by looking for a novel provirus in the affected cells that is a recombinant between endogenous mouse proviruses. Only one exceptional case was found in one of the collaborating laboratories, so that support for direct virus activation by radiation was generally lacking.

Molecular studies on radiation-induced acute myeloid leukaemia in the CBA mouse have included searches for activated oncogenes on chromosome 2, and analysis of haemopoietic growth factor genes. There may be some inactivation with respect to the GM-CSF factor.

The group met in Munich on 19-20 November, with task group (1).

3. Cellular basis of late vascular changes in the areas at risk in the irradiated brain

The problems to be solved are 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 morphological and physiological studies, with multiple sampling times over 1-2 years.

Preliminary results indicate that vasodilatation preceded demyelination. Furthermore, when the deep cortex was irradiated with 20 Gy, no early decrease in vascular density or vasodilatation

developed, but at 12 months post-irradiation both were seen. There was no sign of blood-brain-barrier breakdown, for either large or smaller molecules. However, the antipyrine distribution space in the telencephalon and diencephalon was increased after 15 to 18 months.

In the area at risk, telangiectatic vessels were studied with the electron microscope. Abnormalitites were frequently found at about 18 months after irradiation. The most extensive radiation-induced changes were not in the endothelial cells, but were mainly in the basement membrane and the astrocyte attachment to the blood vessel wall.

A meeting was held on 15 May in Rotterdam.

4. Effects of irradiation on preimplantation mouse embryos

Previous studies had shown that a marked G2-block at the one-cell stage occured when BALB/c zygotes were irradiated with 1 Gy 17-24 h after hCG injection. To elucidate further the underlying mechanism, the pattern of protein synthesis (2D-electrophoresis) was determined. The blocked embryos synthesized proteins as if they were unblocked, except for three polypeptide sets of 30, 35 and 45 kD, which were present in the dividing controls but not in the blocked irradiated zygotes. Considerable changes occurred in the pattern of phosphorylated proteins which are responsible for regulatory processes.

The blocked zygotes displayed significantly more chromosomal abnormalities. However, chromosome aberrations are unlikely to be the cause of the G2-block. Cytogenetic damage was studied after irradiation in the one and two-cell stages. The dose-effect curves were linear up to 0.75 Gy neutrons and up to 1.88 Gy X-rays.

Irradiation at the one-cell stage induced malformations. This effect was dose-dependant without a threshold.

The group met once in the year, at the ICRR in Edinburgh, 19-24 July.

5. Non-stochastic effects of beta-irradiation in mouse and pig skin

The purpose of the experiments was to study the skin reaction of mouse and pig to small area beta sources. Using 1 mm diameter sources of ^{90}Sr and ^{170}Tm, pig skin biopsies examined within 5 days of exposure showed reticular and papillary cell necrosis although the epidermis was still intact. This loss of capillaries and cells from the papillary dermis caused the collapse of the layer after 10-17 days with the loss of the epidermis from 14 days after doses of 200-500 Gy. So the

classical picture of epithelial damage does not hold: damage occurs by endothelial and dermal fibroblast cell death.

Again, in mice exposed to 1 mm ^{90}Sr sources, doses of 300-1500 Gy were needed and again the damage appeared in both dermal and epidermal layers by day 4. Epithelial and follicle mitotic breakdown occurred 2 days later. Regeneration of the damaged area has been studied and shown to be dose-dependent. Further studies at longer time periods are planned, and small ^{244}Cm alpha sources will also be used.

The group met at the CEGB Berkeley Nuclear Laboratories on 27 April.

6. Inter-species comparison of lung clearance

The aim of the first inter-species comparison was to test a model for extrapolating lung clearance measurements from animals to man. The specific objective was to compare the rate of translocation of cobalt from lung to blood, using an aerosol of moderately soluble ^{57}Co oxide particles. Seven species were studied including man. This comparison is now complete and the final report is being prepared for publication. Qualified support was obtained from the study for the particular model being tested: considerable inter-species differences were found in the rates of translocation, lending some uncertainty to the extrapolation of overall clearance to man.

One explanation for the differences noted could be the rate at which particles dissolve after they are ingested by alveolar macrophages. The group met on 7-8 October in Cadarache, where the possibilities were examined for investigating macrophage intra-lysosomal pH as a possible basis for the inter-species differences in clearance. Collaboration with the Karolinska Institute was initiated at the meeting.

Further studies are also being pursued in relation to the interpretation of cobalt oxide retention in the lung.

7. Treatment after incorporation of actinides

A new batch of the decorporating agent LICAM(C) had been prepared for EULEP. This material was subsequently found to contain an esterified form of LICAM and it was nephrotoxic. Nevertheless, it did mobilise plutonium from the liver and the skeleton, although plutonium was concentrated in the kidneys.

A pure form of LICAM(C) has now been prepared and this will be tested to compare its efficacy with the esterified form and with the

compound prepared originally at the Lawrence Berkeley Laboratory, USA.

The decorporation programme was reviewed at the meeting at Reisensburg in March, when it was agreed to give careful consideration to the testing of new chelating agents. Discussions have since taken place with the american group, who have developed a new compound DFO-HOPO, a hydroxylated catechol. Preliminary findings with this material were promising, and a collaborative study by EULEP is under consideration.

A further meeting was held at Mol on 12 December.

8. Stem cell studies after contamination with alpha-emitters

A workshop was held on 29-30 September in Antwerp on "Stem cells in bone and bone marrow after contamination with osteotropic radionuclides". Studies reported there included continuing work on short- and long-term effects of ^{239}Pu and ^{224}Ra on pluripotent colony-forming cells. The radial distribution of CFU-S and GM-CFC in mouse femur was not uniform, but did not vary greatly between 3 and 11 weeks of age. The relative rates of decrease in marrow cell numbers in different bones after ^{224}Ra treatment suggested a rapid migration of proliferating cells between bones during recovery from irradiation. Other studies have examined various stem cells in the Balb/c mouse after tumorigenic doses from ^{226}Ra and ^{241}Am. In long-term studies with ^{224}Ra in CBA mice it appeared that stem cells lying close to the bone gave rise to myeloid leukaemia sooner than those further away.

The effects of alpha-emitters on the microenvironment of stem cells are being assessed using long-term bone marrow cultures. Effects of ^{241}Am have been studied, also the cellular composition of the cultures. Experiments on marrow transplanted to the renal capsule from a donor mouse, in which host stem cells grow, have shown differences between the effects of ^{224}Ra and ^{239}Pu acting on the donor tissue, i.e. on the microenvironment.

9. Metabolism, dosimetry and effects of bone-seeking radionuclides

A number of long-term studies with alpha-emitting radionuclides were reviewed at a meeting in Antwerp on 30 September. One collaborative exercise is investigating plutonium and americium distributions by autoradiography of baboon bones; another has studied ^{226}Ra distribution in bones from beagle dogs. Long-term retention and toxicity of ^{237}Np is being assessed in rats. When ^{239}Pu was given to young rats, changes

in bone structure prevailed, whereas bone and marrow cells were more affected in older rats. In the CBA mouse a comprehensive long-term investigation is comparing ^{239}Pu, ^{241}Am and ^{233}U: average skeletal doses at 448 days from 40 kBq/kg in male mice were 0.48, 0.53 and 0.082 Gy respectively. Autoradiographic work in progress has shown distinctly different distributions on bone surfaces and in macrophages in the bone marrow by 224 days.

Other work has examined pig bone 4 days after injection of ^{226}Ra, thereby giving data comparable to ^{224}Ra. The latter has been used in a long-term study of the dose-response for myeloid leukaemia and osteosarcoma in the CBA mouse. Another experiment with ^{224}Ra in NMRI mice has produced malignant lymphomas in 13% of the animals from marrow doses of 33-200 mGy, provided that the radionuclide was administered twice weekly over 36 weeks.

10. Effects of radiation on the development of the CNS

A meeting to co-ordinate the research of this group was held in Freiburg on 12-13 February.

The purpose of the studies is to assess radiation-induced damage to the developing CNS of man on the basis of model experiments with mice, rats and rabbits. Developmental disturbances were analyzed after external irradiation with X-rays and fast neutrons and after internal exposures by ^{125}I and ^{131}I . Exposure times included various stages of embryogenesis, fetogenesis and the perinatal period. Special attention was paid to the period of corticogenesis which turned out to be the most sensitive "window".

Structural defects are being evaluated at the anatomical and cellular levels by histological and cytological studies including immuno-histochemistry, micrometry and electron microscopy. Biochemical changes of nucleic acids, proteins, lipids and compounds related to energy metabolism are being followed during the critical period of postnatal brain maturation. Special attention is paid to the relevance of cytoplasmic and cellular alterations for neural function and behaviour.

11. Effects of residual radiation injury in dermal and subcutaneous vascular/connective tissue on subsequent skin exposure

The aim of the study is to determine the influence of any residual injury in the dermal vasculature, following an initial exposure

to radiation, on the response of the skin to re-exposure to high energy beta-rays or reactor neutrons. Pig skin was proposed for this study because of its structural similarity to that of man.

An interspecies comparison was carried out using different strains of pig (White pig and Gottinger mini pig), a fixed anaesthetic method (azaperon/etomidat) and a standard irradiation source (22.5 mm diameter ^{90}Sr plaque). The change in incidence of moist desquamation with dose was observed, and the 50% effect level (ED_{50} ± SEM) determined. For the large White pigs this was 36.7 ± 1.3 Gy, and > 33 Gy for the mini pigs. These values were significantly higher than 27.3 ± 0.5 Gy obtained with 2% halothane, 70% oxygen and 28% nitrous oxide. They were also higher than the value of 24.6 Gy obtained after irradiation of the mini pig with ^{60}Co gamma-rays using azaperon/etomidat anaesthesia. At least the first of these differences may be due to hypoxia in the skin induced by the anaesthetic.

There were two working visits between the collaborating laboratories.

IV. Objectives for the next reporting period:

The task group programme will continue as outlined above. In addition progress is anticipated from a task group on radiation effects on the heart and from recently established task groups on (i) radionuclides in the gastrointestinal tract, and (ii) fetal dosimetry and the effects of incorporated radionuclides. Committee activities will include a new external radiation dosimetry intercomparison in 1988 or 1989, and new fascicles of the Pathology Atlas. A symposium is planned on "Radiation effects on the mature and developing central nervous system".

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

VI. Publications:

Symposium on Effects after Combined Exposure to Ionizing Radiation and Chemical Substances, organiser C. Streffer, International Journal of Radiation Biology, 51, 959-1110, 1987.

EULEP Pathology Atlas

EULEP Newsletters 43, 44, 45 and 46

III E

GENETISCHE WIRKUNGEN IONISIERENDER STRAHLEN

GENETIC EFFECTS OF IONIZING RADIATION

EFFETS GENETIQUES DES RAYONNEMENTS IONISANTS

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 detection of radiation damage: Production of antibodies against several radiation-induced lesions (thymine glycols, hydroxymethyldeoxyuridine, UV-type damage, single-strandedness) and development of immunochemical assays based on the antisera obtained.
(ii) sensitive detection of DNA breaks: Development of an alkaline elution method with fluorometric quantitation of DNA, meant to be applicable after in vitro as well as in vivo irradiation.

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

Polyclonal rabbit-antisera were raised against (i) HMU, (ii) poly(dT) treated with OsO_4 to induce thymineglycols and (iii) UV-irradiated DNA, coupled to methylated bovine serum albumin.

Monoclonal antibodies

Immunization of mice was carried out with (i) OsO_4-treated poly(dT), coupled to methylated bovine serum albumin and (ii) HMU and (iii) GpTpTpG containing the thymine dimer; the last two coupled to chicken-γ-globulin as carrier. From spleen cells of these mice, after fusion with 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, 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 and the excess of antibodies 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").

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 damage leading to distortion of the double-helix. This single-strandedness can be amplified enormously by a controlled time-dependent partial unwinding of the cellular DNA by mild treatment with alkali. 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 (assuming 100% single-strandedness if sonication takes place 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. This antiserum recognizes 10^{-15} mol thymine glycol, induced by OsO_4, amongst 10^5-fold excess of unmodified DNA-bases. Thymine glycols appeared to be also detectable after irradiation of DNA in solution with 20-100 Gy τ-rays. 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-τ-rays. The preparation is in progress of more specific monoclonal antibodies against thymine glycol, which may lower the detection level to 1-10 Gy.

Beside the induction of thymine glycol by ionizing radiation, also hydroxylation at the methylgroup of thymine occurs. Possibly, this is a more persistent damage, tolerated by the cells. Polyclonal (rabbit) and monoclonal (mice) antibodies against HMU are already available.

Also UV-type damage is induced by ionizing radiation. Antiserum, raised in rabbits against UV-irradiated DNA, appeared to recognize damage in DNA of human cells exposed to ionizing radiation. With this antiserum, damage was detected on the single-cell level, after doses of ^{60}Co-τ-rays as low as 10 Gy. This technique was improved during this reporting period but it has still to be applied on cells exposed to ionizing radiation.

Immunochemical detection of radiation-induced single-strandedness, amplified by mild alkaline treatment, appeared to be sufficiently sensitive to detect damage induced with 1 Gy of ionizing radiation, both in exposed human blood and in mice (in the white blood cells as well as the bone marrow cells). Damage is still detectable up to 60 min after exposure, indicating that not only the rapid repairable single-strand breaks are 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.

(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. The differential rate of repair in human lymphocytes and granulocytes was confirmed during this report period. The initial rate of repair of SSB *in vivo* in mice white blood cells appeared to be not different from that in murine white blood cells irradiated *in vitro*, but the level of residual SSB 30 min after irradiation was substantially lower *in vivo* than after *in vitro* irradiation. Experiments are in progress for studying the induction and repair of SSB in bone marrow cells and gut cells after irradiation of mice.

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 rat cells than in human cells, whereas unscheduled DNA synthesis (UDS) was only a factor of 2 less. In Chinese hamster cells, accumulation of incision breaks during incubation in the presence of hydroxyurea and β-arabinofuranosylcytosine after UV-C irradiation, was even a factor of 7 lower than in primary human fibroblasts. These results indicate that the extent to which different repair mechanisms are responsible for the removal of damage differs for cells from different species.

(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 not-radioactive DNA. Immunochemical detection with specific antibodies appeared the method of choice.

Rabbits were immunized with UV-irradiated DNA. The antiserum obtained was specific for UV-irradiated DNA; the binding increased with increasing UV-dose. Most of the binding could be prevented by treatment of the UV-irradiated DNA with photoreactivating enzyme and illumination with visible light, indicating that the antiserum has considerable specificity for pyrimidine dimers. The rest-activity could be decreased by affinitychromatography; no activity was present against non-dimer lesions ((6-4)photoproducts). With the antiserum, damage was demonstrated in DNA isolated from human fibroblasts irradiated with 2300 $J.m^{-2}$ UV-B, a dose allowing 37% survival of the cells. Also repair of damage recognized by the antiserum was observed in UV-B-irradiated cells.

Monoclonal antibodies from hybridomas obtained after fusion of spleen cells of mice, immunized with GpTpTpG containing the T-T dimer, also recognized 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. Cultured cells injected with photoreactivating enzyme (isolated from yeast) into the cytoplasma, which subsequently were irradiated with UV followed by photoreactivating light, did not contain thymine dimers, in contrast to similarly treated non-injected cells. These experiments confirm that cultured human cells as such are not able to photoreactivate thymine dimers. XP-cells (group A, D, E and H) also appeared to contain no thymine dimers after UV-irradiation and subsequent microinjection and illumination. Surprisingly, in these cells the low but significant level of UDS is not decreased by injected photoreactivating enzyme and illumination. These data suggest that XP-cells have a delaved low-level UDS (of which the incision step already occurred during the photoreactivation-treatment).

With these antibodies thymine dimers could be detected in situ, by visualization with fluorescent antibodies, in cryostat sections of UV-B-irradiated human skin (200 mJ/cm^2). Clearly, the fluorescence was brightest close to the horny layer and it diminished when going deeper into the skin. However, quantitation of the fluorescent signal is still difficult due to the fact that not all cells are in focus. Nevertheless, it appeared to be possible to obtain a dose-effect relation of cell suspensions prepared from irradiated skin.

IV. Objectives for the next reporting period:

As in the foregoing reporting period, our attention will be focussed on the 2 approaches mentioned before:
(i) Immunochemical 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 in study already, antibodies will be raised against the (6-4)dithymine fotoproduct, induced by UV in DNA.
(ii) Further development of the alkaline elution method, particularly with respect to the detection of UV-endonuclease susceptible sites.

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. P.H.M. Lohman).

VI. Publications:

Friedberg, E.C., C. Backendorf, J. Burke, A. Collins, L. Grossman, J.H.J. Hoeijmakers, A.R.Lehmann, E. Seeberg, G.P. van der Schans and A.A. van Zeeland (1987) Molecular aspects of DNA repair. Mutation Res. 184, 67-86.

Jaspers, N.G.J., L.Roza, W. Vermeulen, A. Eker, R.D.F.M. Taalman, J.H.J. Hoeijmakers and D. Bootsma (1988) In vitro correction of cells from patients with mutagen hypersensitivity. Progress of Int. Congress on DNA repair, Rome (Italy) (in press).

Roza, L., W. Vermeulen, G.P. van der Schans and P.H.M. Lohman (1987) The induction and repair of cyclobutane thymidine dimers in human skin. In: Passchier W.F. and Boschnakovic (Eds) Human exposure to ultraviolet radiation; Risks and regulations, Excerpta Medica International Congress Series pp. 27-32.

Schans, G.P. van der (1988) 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).

Schans, G.P. van der (1988) In vivo detectie van door ultraviolette straling geïnduceerde DNA-schade. Cursusboek Boerhaavecursus Photodermatologie, 1988, Leiden, The Netherlands (in press).

Vijg, J., L. Roza, E. Mullaart and F. Berends (1988) DNA repair in relation to skin aging. Giornale Italiano Di Dermatologia Chirurgica (in press)

Zdzienicka, M.Z., L.Roza, A. Westerveld, D. Bootsma and J.W.I.M. Simons (1987) Biological and Biochemical consequences of the human ERCC-1 repair gene after transfection into a repair deficient CHO cell line, Mutation Res. 183, 69-74.

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. (in press).

Short communications, abstracts...

Roza, L., W. Vermeulen, A.P.M. Eker, J.H.J. Hoeijmakers and P.H.M. Lohman (1987) Immunochemical detection of UV-induced thymidine dimers in individual cells, DNA repair workshop: Molecular Aspects of DNA repair, 1987, Noordwijkerhout, The Netherlands (abstract).

Roza, L. W. Vermeulen and R.A. Baan (1987) Immunochemical detection of UV-induced thymidine dimers in individual cells. Dutch Society for Cell Biology. Cell Biological Basis of Aging, 1987, Veldhoven, The Netherlands.

Schans, G.P. van der, R.A. Baan, R.H. Groenendijk and P.H.M. Lohman (1987) Species and cell type differences influence induction and repair of damage in DNA. DNA repair workshop: Molecular aspects of DNA repair, 1987, Noordwijkerhout, The Netherlands.

Schans, G.P. van der, R.H. Groenendijk and R.A. Baan (1987) Species and cell type differences influence induction and repair of damage in DNA. Dutch Society for Cell Biology. Cell Biological Basis of Aging, 1987, Veldhoven, The Netherlands.

Schans, G.P. van der, R.H. Groenendijk and P.H.M. Lohman (1987) Sensitive detection of single-strand breaks in DNA after in vivo exposure to ionizing radiation to evaluate the effects of protecting agents. 2nd International Conference on Anticarcinogenesis and Radiation Protection, March, 1987, Gaithersburg, USA and: Symposium on Perspectives in Radioprotection, March 1987, Bethesda, USA.

Schans, G.P. van der , R.H. Groenendijk and P.H.M. Lohman (1987 Sensitive detection of single-strand breaks in DNA after in vivo exposure to ionizing radiation: Induction and repair of DNA breaks in blood cells. Proceedings 8th Int. Congress of Radiation Research, July 1987, Edingburgh, UK.

Zdzienicka, M.Z., G.P. van der Schans, A.A. van Zeeland, A. Westerveld and J.W.I.M. Simons (1987). Two UV-sensitive Chinese hamster cell mutants of the same complementation group (UV5 and V-H1) behave differently with respect to repair of UV-damage. DNA repair workshop: Molecular aspects of DNA repair, 1987, Noordwijkerhout, The Netherlands.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Università degli Studi di Milano
via Festa del Perdono, 7
I - 20125 Milano

Contract no.: BI6-E-204-I

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 eukariotic 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 characterization of a yeast protein that cross-reacts with affinity-purified antibodies against recA protein of E. coli.

III. Progress achieved:

METHODOLOGY

In E. coli, recA protein is the key enzyme in recombination. Despite its moderate size (about 39 kDal), this protein is able to find the regions of homology between interacting DNA molecules and to exchange strands between them. The protein must contain several active sites: a) for hydrolysing ATP b) for binding to single-stranded DNA c) for binding to double-stranded DNA d) and e) for binding to two adjacent molecules of recA protein, in order to form long protein filaments. The structural and functional information must be therefore packed in a single polypeptide chain of limited length, leaving limited scope for deviation from an optimized structure. This argues in favour of an evolutionary conservation across philogenetic barriers. We have examined the possibility of detecting analogs of the prokariotic recA protein in eukariotes using polyclonal antibodies as probes.

RESULTS

In 1986 we reported that the yeast S. cerevisiae contains a protein of about 43 kDal (p43) that reacts with affinity-purified anti-recA antibodies. We determined that p43 is present in all strains, whether haploid or diploid, as well as in various strains defective for DNA repair and recombination. Its intracellular concentration increases transiently following irradiation with UV light and during meiosis.

We have purified p43 using as assay its reactivity towards the anti-recA antibodies. Direct purification on a column of immobilized anti-recA antibodies was not successful, indicating that the recognized epitope was not available for binding in the native state. In the first months of 1987 we were able to obtain a highly enriched preparation of p43, and we tested it for DNA-dependent ATPase activity and strand transfer capacity. Neither of these activities were associated with the protein. Depletion or enrichment of p43 in a yeast extract had no effect on its ability to carry out recombination between plasmids carrying different mutations.

Concurrently, we had started the screening with the anti-recA antibodies of a library of yeast DNA in the expression vector λgt11. We had isolated positive clones when we learned that two laboratories in the U.S.A., on the basis of observations similar to ours, had cloned a gene that turned out to code for the small subunit of the enzyme ribonucleotide diphosphate reductase (Elledge and Davis, 1987, Mol. Cell. Biol. **7**: 2783-2793; Hurd et al., 1987, Mol. Cell. Biol. **7**: 3673 3677). Sequence data now indicate that also our clone for p43 contains the same gene. The immunological crossreactivity is due to the last four aminoacids of the small subunit of ribonucleotide reductase, which are identical to the last four

aminoacids of recA protein.

In collaboration with the laboratory of Dr. Johnston at NIMR (London), we have complemented our results on the intracellular concentration of the p43 protein with data on the expression of the corresponding mRNA. As expected, the concentration of the 1.5 kb transcript fluctuates in response to UV irradiation, as well as during the cell cycle and meiosis. A manuscript describing these results is in preparation.

Also in collaboration with Dr. Johnston, we were able to show that the transcription of the yeast DNA polymerase I gene is responsive to DNA damage caused by UV light. The concentration of the 5.2 kb transcript increases for 3 hours after irradiation (50 Jm^{-2}) and returns to basal level after 6 hours. At its peak, the enhancement in transcription level is over 20-fold. These data suggest that polymerase I, the major DNA polymerase of S. cerevisiae, participates in DNA repair as well as DNA replication, although they do not rule out that DNA polymerase II may also play an essential role.

DISCUSSION

At this junction it is clear that we do not have in hand an eukariotic analog of recA protein. We will discontinue this particular line of research and concentrate instead on more general methods for the identification of proteins involved in DNA recombination and repair, as exemplified on the work done on DNA polymerase I and that described in project number 4 (see ahead).

IV Objectives for the next reporting period.

project terminated

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

Dr. Leland Johnston
Laboratory of Cell Propagation
National Institute of Medical Research
The Ridgeway, Mill Hill
London NW7 1AA
United Kingdom

VI. Publications:

Johnston L.H., White J.H.M., Johnson A.L., Lucchini G. and Plevani P. (1987).
The yeast DNA polymerase I transcript is regulated in both the mitotic cell cycle and in meiosis and is also induced after DNA damage.
Nucleic Acids Res. **15**: 5017-5030.

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
Dr. Alessandra Modesti

I. Objectives of the project:

Identification of mammalian proteins involved in DNA recombination and repair.

II. Objectives for the reporting period:

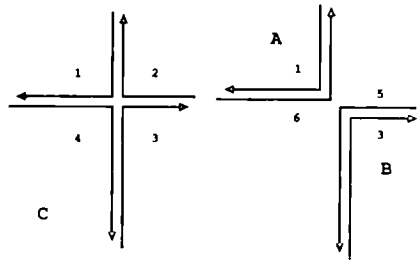
The identification of rat liver proteins that recognize cruciform DNA (Holliday junctions)

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 an artificial Holliday junction by annealing four chemically synthetized oligonucleotides in a four-way branched DNA (molecule **C**). The artificial junction was then labeled with ^{32}P and used as probe in gel retardation experiments. We also built two linear double-stranded molecules (**A** and **B**) by annealing oligonucleotides 1 and 3 with their complementary sequences (6 and 5). The linear duplexes therefore contain all DNA sequences present in the cruciform molecule, and serve as controls for sequence-specific DNA binding.

1 CCCTATAACCCCTGCATTGAATTCCAGTCTGATAA
2 GTAGTCGTGATAGGTGCAGGGGTTATAGGG
3 AACAGTAGCTCTTATTCGAGCTCGCGCCCTATCACGACTA
4 TTTATCAGACTGGAATTCAAGCGCGAGCTCGAATAAGAGCTACTGT
5 GTAGTCGTGATAGGGCGCGAGCTCGAATAAGAGCTACTGT
6 TTTATCAGACTGGAATTCAATGCAGGGGTTATAGGG



RESULTS

A nuclear extract obtained from rat liver was tested for binding to the labeled Holliday junction in the presence of a large excess of duplex salmon sperm DNA as nonspecific competitor. After incubation with the nuclear extract, the mobility of the Holliday junction in polyacrylamide gels was reduced, whereas that of the control duplexes was unaffected.

The putative cruciform binding protein was purified several hundred fold by conventional chromatographic techniques and FPLC and further characterized. The binding of this protein to the labeled Holliday junction is competed by cold cruciform DNA, but not by the control duplexes, single-stranded DNA or nonspecific double stranded DNA. The binding to incomplete Holliday junctions was also investigated: the rat liver protein does not bind at all to DNA molecules composed of oligonucleotides 1 and 2, while it binds weakly to molecules composed of oligonucleotides 1, 2 and 3, which resemble more closely complete Holliday junctions. The rat liver cruciform binding protein also binds to cruciform structures formed by inverted repeats (palindromes) in natural plasmids like ColE1 or pPS11.

No detectable nucleolytic activity towards the artificial Holliday junction or

the cruciform structures extruded by palindromes is associated with the rat liver cruciform binding protein.

DISCUSSION

Our results provide the first demonstration of specific binding to cruciform DNA by a protein from a mammalian source. Several lines of evidence indicate that the binding is structure-specific rather than sequence-specific: (a) probe-DNA complexes were observed in the presence of a 2000-fold excess of nonspecific competitor DNA, (b) binding was not observed with linear duplexes containing sequences identical to those of the cruciform probe, (c) binding occurred with natural as well as synthetic cruciform structures.

Four proteins specific towards cruciform DNA had been characterized so far: three of prokariotic origin and one from the lower eukariote S. cerevisiae. All of them are endonucleases: they cleave the Holliday junction (or any other branched DNA) in order to separate the DNA molecules with crossed-over strands. The rat liver protein, on the contrary, has no nucleolytic activity. Two explanations are possible: (1) the binding protein is a degradation product of a larger polypeptide which has endonuclease activity, or (2) the intrinsic property of the binding protein is actually the recognition and protection of cruciform structures, including the Holliday junctions generated during recombination and repair and the structures extruded from palindromic sequences. The distinction between these two possibilities requires the complete purification of the cruciform binding protein and the availability of antibodies directed against it, but in either case this protein is likely to be an essential component of the cell's recombinational machinery.

IV Objectives for the next reporting period:

The purification of the rat liver cruciform binding protein to physical homogeneity. The production of antibodies directed against it.

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

Dr. Riccardo Cortese
EMBL
Meyerhofstrasse 1
D-6900 Deidelberg
Federal Republic of Germany

VI. Publications:

Bianchi M.E.
Interaction of a protein from rat liver nuclei with cruciform DNA.
EMBO J., in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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: 010-635607

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 no.:

Isolation and characterization of DNA repair genes in mammalian cells.

Head(s) of project:

Dr. A. Westerveld, Dr. J.H.J. Hoeijmakers, Prof.Dr. D. Bootsma

Scientific staff:

Drs. M. van Duin, Dr. N.G.J. Jaspers, Dr. W.J. Kleijer, Drs. M. Koken.
H. Odijk, J. v.d. Tol, J. de Wit, W. Keijzer, W. Vermeulen.

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 (Chinese hamster CHO and V79 mutant cell lines, 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) expression after amplification of the gene in CHO cells. b) effects of site directed mutagenesis in the gene. c) expression in E.coli and production of the gene product.
3. Cloning of human genes correcting the defects in CHO UV-sensitive mutants of the 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

Skin biopsies from patients with a clinical suspicion of a disease associated with mutagen hypersensitivity were sent to us from various European countries. Fibroblast cultures were established from 17 patients and characteristic biochemical or cytogenetical abnormalities were demonstrated for xeroderma pigmentosum (2 out of 5 cases), ataxia telangiectasia (4 out of 9 cases), trichothiodystrophy (1 case), and the Nijmegen breakage syndrome (1 out of 2 cases). Prenatal diagnostic tests using chorionic villi and/or amniotic fluid cells were performed in pregnancies at risk of xeroderma pigmentosum (1 case), ataxia telangiectasia (3 cases) and Cockayne syndrome (1 case).

For a continued genetic survey by complementation analysis the collection of ionizing-radiation sensitive human mutant cells was further expanded. Among 45 cell strains genetically characterized so far, 6 complementation groups were discriminated: four with ataxia telangiectasia (AT) and two with Nijmegen breakage syndrome (NBS). One patient having the clinical signs of both syndromes was assigned to one of the NBS groups, indicating that the two disorders are closely related. In addition, two AT-sibships were identified not showing the radioresistant DNA replication that was considered typical of AT and NBS.

In collaboration with the Leiden group (Simons c.s, see contract no. BI6-E-166-NL) X-ray and UV-light sensitive mutant cell lines were isolated from the Chinese hamster V79 and CHO-9 cell lines. Some of the X-ray sensitive cell lines isolated from the V79 cell line behaved like ataxia telangiectasia fibroblasts with respect to DNA synthesis inhibition after X-irradiation.

2. Isolation of repair genes

a. Molecular characterization of the human DNA excision repair ERCC-1 gene and gene product.

The DNA repair gene ERCC-1 specifically corrects the full spectrum of defective repair parameters of excision deficient CHO mutants belonging to complementation group 2. Elucidation of the complex ERCC-1 gene structure and expression revealed the following features in addition to what has been reported before: 1. Alternative polyadenylation was found to occur generating minor longer ERCC-1 transcripts (3.4 and 3.8 kb in size) with the same coding capacity as the main transcripts. 2. We found another gene within the 3' region of ERCC-1. The 2.6 kb transcripts of this gene overlaps with exon 10 of ERCC-1, terminates within intron 9 and harbors antisense ERCC-1 sequence information. The function of this antisense gene and its relation with ERCC-1 are presently under investigation. This represents -as far as we know- the first example of overlapping transcription units in the human genome.

We have obtained and partially characterized polyclonal antisera directed against the C-terminal half of the ERCC-1 gene product and against the N-terminally located putative nuclear location signal (NLS, using in vitro, synthesized oligopeptides). The anti 'NLS' antiserum showed specific reaction with the nucleus in immunofluorescence, supporting our presumption that this regionhas a nuclear targetting function.

The ERCC-1 polypeptide could be synthesized in vitro by translation of Sp6 ERCC-1 RNA. We are presently utilizing this product for functional studies (eg. DNA binding), and characterization of antisera.

b. DNA transfection of CHO mutants belonging to complementation groups 3 and 6.

The Chinese hamster (CHO) mutant cell lines 27-1 and UV-61 are both sensitive to UV-light and belong to the complementation groups 3 and 6 respectively. As reported previously primary and secondary transformants have been obtained after transfection of 27-1 cells with DNA from repair proficient human cells. From these transformants at least a part of the human gene correcting the repair defect in 27-1 cells has been cloned. This cloned fragment maps on human chromosome 2 and is highly conserved during evolution. (Experiments done in a collaborative project with Drs. G. Weeda in the laboratory of Physiological Chemistry (head Prof. Van der Eb, State University Leiden).

We also obtained primary and secondary transformants in transfection experiments with UV-61. Only in one secondary transformant we can unequivocally identify human sequences. A lambda library has been prepared from the DNA of this transfectant and many recombinants hybridizing with human cot-1 DNA have been identified.

3. Cloning of Drosophila genes homologous with yeast RAD repair genes.

The gene product specified by ERCC-1 exhibits strong homology with the protein encoded by RAD10, a recently cloned repair gene from yeast. This observation might indicate that the excision repair system is strongly conserved during eukaryotic evolution. We utilize this evolutionary conservation for the cloning of human genes by virtue of homology with yeast excision repair genes. We have found that ERCC-1 and RAD10 under very specific hybridization conditions recognize the same fragments on Southern blots containing Drosophila DNAs. Most likely these fragments are derived from the Drosophila equivalent gene. This suggests that Drosophila might function as a kind of 'intermediate' in the cloning process. Similar experiments with different probes of the yeast RAD1 gene indicate that this gene is more strongly conserved than RAD10 and that regions in the Drosophila genome exist with detectable simultaneous homology to different parts of RAD1. Several genomic and cDNA clones have been isolated from Drosophila melanogaster λ-libraries and are now in the process of being characterized:

1. by sequence analysis to determine whether at the nucleotide level and particularly at the deduced aminoacid level extended areas of homology exist.
2. by hybridization with Northern blots and cDNA libraries to verify the codogenic properties of the DNA fragments.

When successful the same approach will be followed for other RAD genes, such as RAD2, 3 and 7, the probes of which have been obtained from Dr. S. Prakash (N.Y.).

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 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.

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

- the Department of Medical Biochemistry, 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 (Dr. A.A. van Zeeland and Dr. J.W.I.M. Simons).
- the Medical Biological Laboratory TNO, Rijswijk (Dr. P.H.M.Lohman).
- the MRC Cell Mutation Unit, University of Sussex England (Prof.Dr. B.A. Bridges).

VI. Publications:

Duin M van, Koken MHM, Tol J van den, Dijke P ten, Odijk H, Westerveld A, Bootsma D, Hoeijmakers JHJ.
Genomic characterization of the human DNA excision repair gene ERCC-1.
Nucl.Acids Res. 1987; 15: 9195-9213.

Hoeijmakers JHJ.
Characterization of genes and proteins involved in excision repair of human cells.
J.Cell Sci. 1987; 6: 111-125.

Keijzer W, Stefanini M, Bootsma D, Verkerk A, Geurts van Kessel AHM, Jongkind JF, Westerveld A.
Localization of a gene involved in complementation of the defect in xeroderma pigmentosum group A cells on human chromosome 1.
Exptl.Cell Res. 1987; 169: 490-501.

Roza L, Vermeulen W, van der Schans GP, Lohman PHM.
The induction and repair of cyclobutane thymidine dimers in human skin.
In: Passchier WP, Bosnjakovic BFM, eds. Human Exposure to Ultraviolet Radiation: Risks and Regulation. Elsevier Science Publishers B.V. (Biomedical Division) 1987; 27-32.

Zdzienicka MZ, Roza L, Westerveld A, Bootsma D, Simons JWIM.
Biological and biochemical consequences of the human ERCC-1 repair gene after transfection into a repair-deficient CHO cell line.

Mutat.Res. 1987; 183: 69-74.

Bootsma D, Koken MHM, van Duin M, Westerveld A, Yasui A, Prakash S, Hoeijmakers JHJ.
Homology of mammalian, Drosophila, Yeast and E.coli repair genes.
In: Fielden EM, Fowler JF, Hendry JH, Scott D. eds. Radiation Research. Proceedings of the 8th Int.Congress of Radiation Research- Edinburgh. Taylor and Francis, London 1987; 412-417

Hoeijmakers JHJ, Odijk H, Westerveld A.
Differences between rodent and human cell lines in the amount of integrated DNA after transfection.
Exp.Cell Res. 1987; 169: 111-119.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no : BI6-E-142-UK

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

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

Prof. B.A. Bridges
MRC Cell Mutation Unit
University of Sussex
Falmer
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Telephone number: 0273-606755 211

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:

To:

a) to maintain expansion of the culture collection
b) to continue the cellular immortalization programme and characterise the products
c) to develop alternative strategies for transfer of resistance genes to human cells
d) to characterise flanking sequences
e) to analyse the radiation resistance of bladder-derived tumour cells
f) to select an alkylation damage repair proficient derivative of a mer^- cell line

III. Progress achieved:

(a) Expansion of the cell culture collection

Cell cultures have been accumulated in the form of fibroblasts (24), amniocytes (3), skin biopsies - developed into fibroblast cultures (31) and T-lymphocyte lines (10). Amongst these are cells for routine diagnosis of DNA repair defective diseases and cells from a population of individuals exposed to ionising radiation.

(b) Immortalization and characterisation of cell lines

We have now established a set of 10 immortalised cell lines following transfection with pSV3gpt or pSV3neo and selection for the dominant marker (mainly gpt). Cells from one normal, two Cockayne syndrome, two ataxia-telangiectasia (A-T) probands, three A-T heterozygotes (one with neo selection), one hypogammaglobulinaemia (46BR) and one trichothiodystrophy are now designated immortalised. Large stocks of these cells, and others which are in progress towards immortalization have been established. Stable 6-thioxanthine-resistant derivatives of a pSV3gpt-immortalised A-T heterozygote have been established. The radiation resistance conferred by transformation has been confirmed, as has the lack of any major enhancement of the immortalization step by classical mutagens recorded in the previous report.

Experiments, analogous to those with ionising radiation, are in progress to determine the stage at which those immortal cell lines which become mex^- lose O^6-alkyltransferase activity.

(c) Alternative strategies for transfer of resistance genes to human cells

Plasmids based on the Epstein-Barr virus have the ability to exist extrachromosomally in human cells and we are currently evaluating the use of an EB-based cosmid library for gene transfer. The critical questions are the amount of rearrangement that occurs during transfection, the number of independent library members that can persist stably in a transfected cell, and whether exogenous DNA within an EB-based plasmid is more stable than such DNA integrated into a chromosome.

The active site of the ligase gene is highly conserved in evolution, and we have constructed oligonucleotides, corresponding to the yeast ligase active site. We are currently probing Drosophila and other libraries for clones showing homology to this active site.

(d) Radiation resistance of human bladder and testicular derived tumour cell lines

A comparison of the gamma-ray sensitivity of these two classes of cell reveal that 5 cell lines derived from the testis are more radiosensitive than 4 bladder-derived cell lines. The bladder cells had radiation sensitivities (Do=1.82Gy) similar to that of an SV40 transformed normal cell line (MRC5V1). The testicular cells were hypersensitive (Do=1.36Gy) but not as sensitive as an SV40 A-T cell line (AT5BIVA, Do=0.98Gy). In contrast to the situation in A-T cells, there was no association between a lack of post-irradiation inhibition of DNA synthesis and radiosensitivity in the testicular cell lines.

(e) Progress towards the molecular cloning of Cockayne syndrome and 46BR

UV-resistant transfectants from a gpt-immortalised Cockayne syndrome cell line (CS3BE1GN) only maintain resistance on weekly subculture with a dose of 254nm UV light which kills the cell line. The transient nature of this response precludes the use of DNA from these cells for secondary transfection.

3-Aminobenzamide-resistant secondary transfectants have been obtained following genomic transfer with 46BR cells. Such cells are proving extremely difficult to maintain in culture and until the tissue culture characteristics of them can be improved, study of their properties is inhibited.

(f) Selection of an alkylation damage repair-proficient derivative of a mex$^-$ cell line

Two methylnitrosourea-resistant derivatives of the mex^{-1} cell line MRC5V1 have been isolated and their cross-resistance to a number of agents is being studied.

IV. Objectives for the next reporting period:

(a) to maintain expansion of the culture collection
(b) to continue the cellular immortalization programme
(c) to study loss of O^6-alkyltransferase during the process of immortalization
(d) to maintain progress towards the molecular cloning of Cockayne syndrome and 46BR genes
(e) to develop alternative strategies for transfer of resistance genes to human cells
(f) to compare the radiation responses of T-lymphocytes and fibroblasts and to analyse their reproducibility

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:

Dean, S. W. (1987) Some aspects of glutathione metabolism in ataxia- telangiectasia fibroblasts. Int. J. Radiat. Biol., 52, 43-48.

Green, M. H. L., J. E. Lowe, C. F. Arlett, S. A. Harcourt, J. F. Burke, M. R. James, A. R. Lehmann, and S. M. Povey (1987) A gamma-ray resistant derivative of an ataxia-telangiectasia cell line obtained following DNA-mediated gene transfer. J. Cell Science, Suppl. 6, 127-137.

Lehmann, A. R., C. F. Arlett, J. F. Burke, M. H. L. Green, M. R. James, and J. E. Lowe (1986) A derivative of an ataxia-telangiectasia (A-T) cell line with normal radiosensitivity but A-T like inhibition of DNA synthesis. Int. J. Radiat. Biol., 49, 639-643.

Parris, C. N., C. F. Arlett, A. R. Lehmann, M. H. L. Green, and J. R. W. Masters (1988) Differential sensitivities to gamma radiation of human bladder and testicular cell lines. International Journal of Radiation Biology, in press.

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 (1) 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) Continued analysis of Gpt^- cell lines derived from C10 and E2 by UV- and gamma-irradiation, and EMS treatment. Analysis of the structure of amplified and extrachromosomal gpt sequences in Gpt^- derivatives.

(b) Production and analysis of spontaneous and radiation-induced mutants in the lacZ gene of EBV shuttle vectors.

(c) (i) Measurement of mutant frequencies in circulating T-lymphocytes from families segregating DNA repair defects
(ii) Molecular analysis of the hprt and T-cell receptors (Ti) alpha and beta chain genes in mutant lymphocytes.

III. Progress achieved:

(a) Integrated gpt gene

In the previous report we described the characterisation of spontaneous gpt$^-$ derivatives of cell lines containing a single integrated copy of the gpt gene. A variety of different alterations, including deletions, amplifications, methylation and phenotypic switching were detected. We have since discovered that the apparently amplified sequences resulted from spurious contamination. We have carried out a similar analysis of 41 UV-induced and 18 EMS-induced Gpt$^-$ derivatives. The types of alteration are shown in the Table. In most cases inactivation of the gene resulted from alterations of gene expression rather than from mutations. In the majority of cases this inactivation of gene expression was caused by methylation.

Types of Alteration (no. found/no. tested)

	Deletion or rearrangement	Methylation	None detected (point mutation?)
Spontaneous	5/6	3/3	0/3
UV-induced	1/41	10/11	0/17
Spontaneous	1/6	0/2	1/4
EMS-induced	4/18	3/5	0/7

(b) Shuttle vectors

Our Epstein-Barr virus shuttle vector was described in the previous report. We have generated several mutants in normal cells following UV irradiation. In only one out of 20 ataxia-telangiectasia derivatives containing the shuttle vector was the plasmid maintained extrachromosomally, and attempts to recover the DNA from this line were not successful.

(c) (i) Measurement of mutant frequencies

We have continued to measure the frequency of mutants resistant to 6-thioguanine in the circulating T-lymphocytes from normal or repair-deficient individuals. Elevated mutant frequencies when compared with age matched normals are confirmed for ataxia-telangiectasia patients. The heterozygotes are not significantly different from normals. Elevated mutant frequencies were also recorded from excision-proficient and excision-defective xeroderma pigmentosum patients and from individuals suffering from Cockayne syndrome.

(ii) Molecular analysis

6-thioguanine resistant mutants and control unmutated clones have been isolated from normal and DNA repair defective individuals. We have commenced a study of these cultures and HPRT enzyme levels have been determined and Southern blotting performed with a limited number

IV. Objectives for the next 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 receptors (Ti) alpha and beta chain genes in mutant lymphocytes.

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:

Friedberg, E. C., C. Backendorf, J. Burke, A. Collins, L. Grossman, J. J. H. J. Hoeijmakers, A. R. Lehmann, E. Seeberg, G. P. Van Der Schans, and A. A. Van Zeeland (1987) Molecular aspects of DNA repair. Mutation Research, 184, 67-86.

Gebara, M. M., C. Drevon, S. A. Harcourt, H. Steingrimsdottir, M. R. James, J. F. Burke, C. F. Arlett, and A. R. Lehmann (1987) Inactivation of a transfected gene in human fibroblasts can occur by deletion, amplification, phenotypic switching, or methylation. Mol. Cell. Biol., 7, 1459-1464.

Lehmann, A. R. (1987) Cockayne's syndrome and trichothiodystrophy: defective repair without cancer. Cancer reviews, 7, 82-103.

Muriel, W. J., J. Cole, and A. R. Lehmann (1987) Molecular analysis of ouabain-resistant mutants of the mouse lymphoma cell line L5178Y. Mutagenesis, 2, 383-389.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Medical Research Council
20 Park Crescent
GB - London W1N 4AI

Contract no.: B16-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:

To assess the effectiveness of the Robertsonian translocation, Rb(1.3)1Bnr, with both arms marked for detecting chromosome 1 and 3 non-disjunction using the Rb tester method.

III. Progress achieved:

1. Methodology

1. The effectiveness of doubly-marked Rb1Bnr heterozygotes as tester animals for detecting chromosome 1 and 3 non-disjunction and loss events in chromosomally normal mice was ascertained by screening for loss events, initially in females. As in previous studies, leaden (ln) was employed as the chromosome 1 marker but, here, in addition, chromosome 3 was marked with matted (ma). Chromosome loss was induced by X-rays. Unmarked females were placed with ln ma males that were heterozygous for Rb1Bnr for one week following 4 Gy X-irradiation and the progeny scored for ln and ma.

2. When the results of the above experiment became known reciprocal crosses were set up to screen for the chromosome 1 and 3 loss events in males. The 4 Gy dose was again used and since the irradiated chromosomally normal males were left with marked tester females for only one week all induced loss events derived from treated spermatozoa.

2. Results

1. 612 females were employed in the first experiment and most (518) became pregnant and yielded young for phenotypic classification. With the mean litter size (5.63) also being acceptably high, the test system may be regarded as a practical one.

Of 2915 progeny born, 2750 were reared to an age (5 days) when the ln phenotype could be distinguished and 2687 reached weaning age (20 days) when classification for ma became possible. The relatively high pre-weaning losses were due to exceptional events unrelated to the experiment. 6 ln young (0.22%) were detected and all survived to an adulthood when their genotypes could be verified. From this result the frequency of chromosome 1 loss among the gametes of the irradiated females was calculated (3.02%; 95% confidence limits, 1.20 and 5.69) and found to be in very good agreement with earlier data (3.50%; 95% confidence limits, 1.54 and 6.19). In contrast to the chromosome 1 response detected with ln, no chromosome 3 loss was detected with ma. The difference between the ln and ma data is statistically significant ($P = 0.001$).

2. The test for chromosome 1 and 3 loss following male irradiation have not yet provided informative results.

3. Discussion

Preliminary studies using the Rb complementation test had indicated that the frequency of chromosome 3 non-disjunction in Rb1Bnr heterozygotes (19.2 ± 1.7%) was very similar to that for chromosome 1 (22.4 ± 1.6%). The failure to detect any indication of chromosome 3 loss in the present experiment using Rb1Bnr with the Rb tester method is therefore unexpected and cannot be explained by the non-complementation phenomenon that was responsible for the failure to detect chromosome 6 loss following female irradiation. It will be of interest to determine whether chromosome 3 loss can be detected following the irradiation of males. Further studies with females are also warranted.

IV. Objectives for the next reporting period:

1. To finalise the tests with irradiated males.

2. To obtain further data with females.

3. To investigate the effectiveness of Rb(11.13)4Bnr with both arms marked for detecting chromosome 11 and 13 non-disjunction using the Rb tester method. This translocation has been extensively studied using the Rb complementation method.

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

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VI. Publications:

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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 more validly be extrapolated to man.

II. Objectives for the reporting period:

1. To investigate further the cell stage sensitivity differences of spermatogonial stem cells to specific locus mutation and translocation by X-rays.

2. To investigate the basis of the X-ray fractionation effects observed in C3H/HeH mice.

3. To investigate the influence of different priming doses of TEM upon X-ray-induced translocation yields in combined TEM-X-ray treatments with 24 h intervals.

III. Progress achieved:

1. Methodology

1. The specific locus mutation response was determined following a) a combined 500 mg/kg HU (2 doses, 3 h interval) + 6 Gy X-ray treatment (24 h interval), b) a combined 750 mg/kg HU dose + 6 Gy X-ray treatment (<½ h interval), c) a combined 2.0 mg/kg TEM dose + 6 Gy X-ray treatment (3 h interval), and d) HU treatment alone (2 500 mg/kg doses, 3 h interval). Allelism tests were carried out on all specific locus mutations. Progeny carrying dominant mutations were also kept and subjected to breeding tests.

2. The translocation response of C3H/HeH spermatogonial stem cells to 24 h fractionated 3 + 3 Gy, 1 + 5 Gy, 4 + 4 Gy and 1 + 7 Gy X-ray treatments were investigated. The responses to single 6 and 8 Gy doses were also obtained.

3. The translocation response of the spermatogonial stem cells of C3H/HeH x 101/H F_1 hybrid mice to 6 Gy X-rays 24 h after 2 mg/kg, 3 mg/kg and 4 mg/kg TEM pre-treatments were investigated. Control groups were given single doses of 4 mg/kg TEM and 6 Gy X-rays

In each translocation experiment the response was assessed by the standard method of screening spermatocytes for multivalent associations at diakinesis/MI 3 or more months following mutagenic treatment.

2. Results

1. Whereas no specific locus mutations were detected among 7367 progeny in the HU group, 5 were found among 6737 in the HU-X-ray, 24 h interval group (frequency, 10.06×10^{-5}/locus/gamete), 5 in 6764 in the HU-X-ray, <½ h interval group (frequency, 10.56×10^{-5}) and 3 in 6685 in the TEM-X-ray, 3 h interval group (frequency, 6.41×10^{-5}). 6 dominant mutations were also recovered.

2. The translocation frequencies obtained in C3H/HeH mice following single 6 and 8 Gy and 24 h fractionated 3 + 3 Gy, 1 + 5 Gy, 4 + 4 Gy and 1 + 7 Gy X-ray doses were 6.50%, 4.78%, 8.75%, 13.50% and 12.47%, respectively.

3. The translocation yields from the hybrid mice following 4 mg/kg TEM, 6 Gy X-rays, and combined TEM (2, 3 and 4 mg/kg)-X-ray treatments were 0%, 9.33%, 17.21%, 19.14% and 17.1%, respectively.

3. Discussion

1. The failure to detect specific locus mutations following HU treatment alone and the absence of any increase over a 6 Gy response (13×10^{-5}) when HU was given immediately before 6 Gy irradiation indicates that the greatly enhanced mutation yield previously obtained with a combined HU-6 Gy X-ray (3 h interval) treatment derives neither from mutagenic action by HU nor from an interaction between the two agents in the treated cells. Rather the data support the conclusion that HU treatment several hours prior to irradiation enhances the proportion of cells in G_1 by killing S phase cells, and that G_1 is the stage of the spermatogonial stem cell cycle most sensitive to specific locus mutation induction by X-rays. The sub-6 Gy mutation response with the TEM pre-treatment (3 h interval) suggests that the chemical tends to act upon the stage of the cell cycle sensitive to X-ray induced mutation (G_1).

2. The translocation yields from C3H/HeH spermatogonial stem cells exposed to single and 24 h fractionated X-ray exposures were generally lower than obtained with C3H/HeH x 101/H F_1 hybrid mice but this could be an artefact of observer scoring differences. However, the responses to the fractionated treatments relative to that for single doses also differs from those with the hybrid mice and may relate to the previously observed differences in the relative amounts of stem cell killing. An altered radiation response of stem cells surviving the first exposure is indicated.

3. The translocation response of hybrid mouse spermatogonial stem cells to 6 Gy X-rays increased with all TEM pre-treatments, but was not dependent upon TEM dose.

IV. Objectives for the next reporting period:

1. To investigate further the cell stage sensitivity differences of spermatogonial stem cells to specific locus and translocation induction by X-rays.

2. To investigate further the sensitivity of spermatogonial stem cells of 101/H and other mouse strains to killing and translocation induction by X-rays.

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

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VI. Publications:

1. Cattanach, B.M. and Rasberry, C. Genetical effects of combined chemical-X-ray treatments in male mouse germ cells. Int. J. Radiat. Biol. 51: 985-996 (1987).

2. Cattanach, B.M. and Rasberry, C. A new curtailed mutation. Mouse News Letter 77: 122 (1987).

3. Cattanach, B.M. and Rasberry, C. A new T allele. Mouse News Letter 77: 122-123 (1987).

4. Cattanach, B.M. and Rasberry, C. A dominant mutation affecting the feet. Mouse News Letter 77: 123 (1987).

5. Cattanach, B.M., Lyon, M.F., Peters, J. and Searle, A.G. Agouti locus mutations at Harwell. Mouse News Letter 77: 123-125 (1987).

6. Rasberry, C. and Cattanach, B.M. A new polydactyly mutation. Mouse News Letter 78: 50 (1987).

7. Cattanach, B.M. and Rasberry, C. New translocation; T(4;15)46H. Mouse News Letter 78: 51 (1987).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 - 91190 Gif-sur-Yvette

Telephone number: 6-907 78 28

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.:

Induction of SOS from procaryotes to higher eucaryotes

Head(s) of project:

Raymond DEVORET

Scientific staff:

R. Devoret, A. Bailone, P. L. Moreau, M. Dutreix, J. Angulo, J. Celerier, S. Sommer, M. Pierre, D. Bouillon, G. Coste.

I. Objectives of the project:

1) During the preceding contract, we demonstrated that RecA protein protects chromosomal DNA against most physical (radiations) and chemical carcinogens. We are now investigating the molecular basis of RecA protein protective action using bacteria as a test system.

2) We are extending our study to identify structural or functional analogs of RecA protein in higher eucaryotes such as rat cells.

3) As RecA protein protects the cells by being induced as an SOS gene product, we aim at determining the nature of the SOS signal. What is the mechanism of activation of RecA protein that entails the derepression of the SOS genes?

II. Objectives for the reporting period:

Our objectives are:

1) to isolate recA mutants with a split phenotype, that is, mutants displaying only one deficiency at a time. We want to correlate the deficiency with a physical map of mutations in the recA gene so as to define the enzymatic domains of the protein.

2) to identify an analog of RecA protein in rat cells that cross-react with antibodies raised against RecA protein of E. coli.

3) to determine the nature of the SOS signal produced by radiations.

4) to characterize the mechanism of PsiB polypeptide as an antagonist of the activation of RecA protein.

III. Progress achieved

1- Novel split phenotype mutants of RecA protein

(Dutreix, Bailone, Moreau and Devoret)

1. Methodology

In order to understand the diverse functions of RecA protein, such as the co-protease and recombinase functions, and to determine which of them ensure a radio-protective effect, we set to isolate new recA mutants.

The recA gene of Escherichia coli was cloned into the miniF plasmid. The advantage of using miniF plasmid as a vector is that the recA gene dosage is one per chromosome so that the recipient cell has a normal cellular level of RecA protein. Mutations in the recA gene have been isolated by localized mutagenesis. All the mutations obtained were sequenced.

2. Results

We have obtained recA mutants displaying split phenotypes. We have 3 mutants whose specificity for repressor cleavage has been modified so that they recognize one repressor specifically. Such mutants should allow us to determine the RecA protein domains that control the cleavage of repressors. We have found mutants of recA that are protease constitutive and lack recombinational activity, suggesting that the two main biological activities of RecA protein (SOS induction and homologous recombination) can be ascribed to separate domains of the protein.

3. Discussion

The relationship between the physical domains and the diverse functions of RecA protein will be compared to the theoretical three- dimensional model proposed by Dr. A.J. Clark (Berkeley).

2- Analogs of RecA protein in mammalian cells

(Angulo, Bertolotti, Moreau and Devoret)

1. Methodology

Antibodies raised against RecA protein of E. coli were used to characterize an immunological analog in rat 3T3 cells.

2. Results

We have found a protein inducible by ultraviolet light and mitomycin C. The protein has a molecular weight of 120 kilodaltons and is in the cell nucleus.

3. Discussion

A protein of the same molecular weight has just beeen found by Dr. Rich (MIT). We will determine whether the two proteins are similar.

3- A mechanism for the activation of RecA protein
(Moreau)

1. Methodology

RecA protein must be activated in order to act as a coprotease in the cleavage of repressors such as LexA protein or cI protein of a lambdoid phage. Activation of RecA protein is primarily controlled by the protein itself and also by other proteins such as RecF and single- strand binding protein (SSB).

2. Results

The single-strand binding protein (SSB) has been shown to determine the conversion of RecA protein from an initial coprotease activity to a recombinase activity, this being also due to the property of RecA protein to polymerize on single- strand DNA. RecF protein may favor the conversion of RecA protein to a recombinational activity by antagonizing SSB protein bound to single-stranded DNA.

3. Discussion

RecA protein activation appears to be controlled by a few proteins. A new protein called PsiB (12 kDa) has been shown (see below) to antagonize the activation of RecA protein, thus preventing SOS induction.

4-Plasmid PsiB protein prevents activation of RecA protein
(Bagdasarian, Bailone, Bagdasarian, Sommer, Celerier and Devoret)

1. Methodology

Since plasmid F DNA is transfered as a single-strand, it seems paradoxical that transfer of plasmid F DNA during bacterial conjugation does not induce an SOS signal. We set to investigate whether there was a plasmid function that would prevent the formation of an SOS signal during conjugation in the hope that we would find an inhibitor of SOS induction.

2. Results

We found that plasmid R6-5, an analog of plasmid F, carries a gene called psiB whose product inhibits the induction of sfiA and prophage lambda in a reA441 mutant at 42^0 and in a $recA^+$ host after treatment with nalidixic acid. The plasmid SOS-inhibitor gene is situated near oriT, the origin of plasmid conjugational transfer. The PsiB function affects in vivo the generation of an SOS signal by counteracting the activation of RecA protein.

3. Discussion

The PsiB protein may have a direct action on RecA protein or may compete with it at the replication fork. We are investigating the two possibilities. The use of PsiB polypeptide that counteracts activation of RecA protein provides us with a new tool to discriminate between the sites of action of RecA protein. Some proteins are involved in DNA metabolism while some others maintain the chromosome structure. PsiB protein may participate in a chromatin-like complex that regulates the access of RecA protein to single-stranded DNA.

IV. Objectives for the next reporting period

1. Anti-RecA protein.

We want to identify the mechanism whereby PsiB polypeptide counteracts the action of RecA protein. We will determine the site at which RecA protein is activated whether at the replication fork or at the lesions or at both sites.

2. Analogs of RecA protein in mammalian cells.

We want to identify and characterize analogs of RecA protein in mammalian cells.

3. Correspondance between recA mutations and protein domains.

The sequence of the recA mutations will be correlated with the changes in phenotypes of the recA mutants.

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

Pr Michael Bagdasarian, Michigan Biotechnology Inst., PO Box 27609, LANSING, MI 48909, USA

VI Publications:

Angulo J, Schwenke J, Moreau PL, Moustacchi E, Devoret R (1985) A yeast protein analogous to Escherichia coli RecA protein whose cellular level is enhanced after irradiation. Molec. Gen. Genet. 201:20-24

Bagdasarian M, Bailone A, Bagdasarian MM, Manning PA, Lurz R, Timmis KN, Devoret R (1986) An inhibitor of SOS induction specified by a plasmid locus in Escherichia coli. Proc. Natl. Acad. Sci. USA 83:5723-5726

Bailone A, Brandenburger A, Levine A, Pierre M, Dutreix M, Devoret R (1984) Indirect SOS induction is promoted by ultraviolet light-damaged miniF and requires the miniF lynA locus. J. Mol. Biol. 179:367-390

Bailone A, Sommer S, Devoret R (1985) Mini-F plasmid induced SOS signal in E coli is recBC-dependent. Proc. Natl. Acad. Sci. USA 82:5973-5977

Brandenburger A, Bailone A, Levine A, Devoret R (1984) Gratuitous induction. J. Mol. Biol. 179:571-576

Devoret R (1983) Inducible responses to DNA damage. In: Friedberg EC, Bridges BA (eds) Cellular Responses to DNA damage, pp 571- 576

Devoret R (1987) Molecular aspects of genetic recombination. In: Michod RE, Levin BR (eds) The Evolution of Sex: An Examination of Current Ideas, Sinauer Ass., pp 24-44

Devoret R, Pierre M, Moreau PL (1983) Prophage Ø80 is induced in Escherichia coli K12 recA430. Molec. Gen. Genet. 189:199-206

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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Moreau PL, Roberts JW (1984) RecA protein-promoted lambda repressor cleavage: Complementation between RecA441 and RecA430 proteins in vitro. Molec. Gen. Genet. 198:25-34

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RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
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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.: 1

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.
AURIAS A.	MULERIS M.
COUTURIER J.	PRIEUR M.
DUTRILLAUX B.	VIEGAS-PEQUIGNOT E.

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:

Analysis of the chromosomal rearrangements (deletions + acentrics and isocentrics , translocations, dicentrics, inversions and other occurring either spontaneously or after irradiation of exposure to chemical mutagene).

III. Progress achieved:

1) Methodology :

Human lymphocyte cultures were performed, during 48 and 72 h, and preparations were treated for R-banding. Each metaphase was analysed by 2 observers independently. Fractions of 48 h cultures were irradiated in Go-phase by γ-rays at the following doses : .0,5, .1, .2 and .5 Gy.

2) Results and Discussion

All the material, i.e. about 10.000 metaphases, was accumulated for the development of the program in human controls.

During this year, two types of anomalies were studied :

1) The deletions, and the resulting acentrics and isoacentrics. The main new results obtained can be summarized as follows

- spontaneous or induced deletions do not occur at random.
- they are the main rearrangement induced by low dose radiations, and are replaced by interchromosomal exchanges at higher doses.
- the isoacentrics, resulting from the duplication of acentrics, have a strong tendency to be formed after breakage in juxtacentromeric heterochromatin.

When they result from breakage of euchromatic regions, they are not distributed at random since they frequently occur at place where centromeres have existed in other primate chromosomes.

It is assumed that intercalary structures exist in human chromosomes which have conserved some of the properties of heterochromatin (reference N° 1).

2) Other chromosome rearrangements

Two types and perhaps a third one exist. One type is composed of translocations and inversions resulting from breaks in 7p14, 7q35, 14q12 and 14qter. These translocations were detected previously by several authors, but our result give their frequency and their relationship with aging.

Unexpectedly, they are more frequent in new borns (.005) than in adults (.002). They are not induced by mutagenes, or at least very rarely. They are presumed to occur in utero and to correspond to abnormal rearrangements of immunoglobulin family genes.

The second type of abnormalities include all rearrangements which do no affect the 4 bands cited above.
Their frequency clearly increases with aging :
.003 in new borns, .014 in young adults and .018 in old adults. Their dependance from age corresponds to that of deletions.

The third type is intermediary between the 2 first.
It is composed by rearrangement of one of the 4 bands cited above with another. It may increase with aging but our sample remains too small to be significant.

On the whole, the frequency of all the rearrangements (except those of the chromatid type and gaps) was found to be : .018 in new borns, .029 in young adults and .042 in old adults.
Only those increasing with aging can be regarded as possibly induced by mutagens.

IV. Objectives for the next reporting period:

a. Study of chromosomal rearrangements in cultures irradiated at low doses.

b. Study of chromosomal rearrangements induced by chemical mutagenes, and comparison with those radiation induced.

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

VI. Publications:

1. Dutrillaux B., Al Achkar W., Aledo R., Aurias A., Couturier J., Dutrillaux A.M., Flüry-Herard A., Gerbault-Seureau M., Hoffschir F. Lamoliatte E., Lefrançois D., Lombard M., Mamuris Z., Muleris M., Prieur M., Ricoul M., Sabatier L. and Viegas-Péquignot E. Isoacentric and isocentric chromosomes originating after deletions of human chromosomes. Hum. Genet. 76 : 244-247 (1987).

2. Prieur M., Al Achkar W., Aurias A., Couturier J., Dutrillaux A.M., Dutrillaux B., Flüry-Herard 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. Human Genet. (In press).

Title of the project no.: 2

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 : t(14;14), inv(14), and t(X;14).
Effect of caffeine in the cell cycle in Fanconi anaemia.
Chromosome study in skin cancers from xeroderma pigmentosum patients.
Cytogenetic study of the sensibility of normal donors to alkylating agents.
Cytogenetic study of the patients affected by so-called secondary leukemias.

III. Progress achieved

1) Methodology

The cytogenetic study of the patients affected by various precancerous diseases has been pursued :

- one thousand karyotypes from 10 independent patients affected by xeroderma pigmentosum are under study, as well as 1300 karyotypes obtained after in vitro mutagenesis by alkylating agent. The number of patients affected by atexia telangiectasia studied is now 75.

2) Results and discussion

The effect of caffeine on the cell cycle in Fanconi anaemia has been studied and has shown that the slowing down is released by caffeine. In patients affected by retinoblastoma, with and without microdeletion of chromosome 13, no increased radiation sensitivity was found after irradian by γ -rays at S- and G2-phases.

The study by in situ hybridization of the clonal anomalies from ataxia telangiectasia has been pursued (ref. 1, 2). The distal breakpoint on chromosome 14 has been found localized between probe D14S1 and Pi gene. In the tct(14;14) carrier clone, a correlation between this abnormal karyotype and a peculiar T-cell population has been established. A cytogenetic and molecular study of a patient affected by several simultaneous lymphomas has been performed. These results will be published soon.

One thousand karyotypes from ten independent patients affected by xeroderma pigmentosum have been studied. A high rate of chromatid breaks and gaps has been observed. Several skin carcinomas ascertained in these patients have been studied (ref. 3).

More than one thousand mitoses obtained after in vitro mutagenesis of lymphocytes by alkylating agents have been accumulated. A preferential involvement of R-band rich regions in the rearrangements has been observed. In particular, chromosomes 5, 7, 11 and 17 are frequently involved. The effects of alkylating agents in vivo are under study.

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 (induced) leukemias.

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

VI. Publications:

1. Stern M.H., Zhang F., Griscelli C., Thomas G. and Aurias A. Molecular characterization of different ataxia telangiectasia T-cell clones. Hum. Genet. 78 : 33-36 (1988).

2. 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. (In press).

3. 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. (In press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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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 qualititative 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: VIEGAS-PEQUIGNOT Evani
HOFFSCHIR Françoise
SABATIER Laure
AL ACHKAR Walid

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:

- 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.
- Study of the distribution of the breaks in an unusual karyotype : that of Ateles.

III. Progress achieved:

1) Methodology

Chromosome aberrations were studied after lymphocyte cultures and R-banding. On some experiments, a BrdU incorporation technique was associated to a heat denaturation in order to obtain a R-banding and to evidence the number of cell division.

Results and discussion

1) The work developed on Ateles chromosomes was achieved. It clearly showed that the rate of breakage of large chromosomes does not follow the rules established in the hypothesis of a rate of breakage proportionnal to chromosome length. Especially for inversions, large chromosomes are too rarely affected. This observation and other arguments we previously developed let suppose that the interphasic organization of chromosomes into domains plays an important role in the non random character of chromosomal mutagenesis (ref. 1).

2) We have shown that the lesions induced by Neon ions (250 Mev/u and 125 Mev/u at synchrocyclotron Saturne) are more complex than those induced by γ -rays at the same dose. The number of breaks is higher per abnormal metaphase and per rearrangement on the average. For a given ion, at the same fluence, the complexity increases with let. The existence of a high rate of such rearrangements could result in a large difference between the number of dicentrics scored by standard techniques and the number of aberrations effectively induced (ref. 2).

3) The transmission of chromosome rearrangements was studied by a method which allowed us to distinguish between cell survival and slowing down of the cell cycle.

It is shown that the frequency of the rearrangements, at each cell division, depends not only on the type of rearrangement but also, and may be above all, on the association of several rearrangements in the same cell.

4) The influence of both time and cell cycle on radiation-induced chromosome lesions was studied. It is shown that both parameters are of importance. Cell cycle is more important for the type of rearrangement, and the time elapsed between irradiation and harvesting is more important for the number of lesions.

The high sensitivity of cells two hours before harvesting is observed for cells irradiated during late S phase as well as during G2-phase. Thus, if it corresponds to the previously described transition point, it must be considered that this transition point is not specific to G2-phase.

IV. Objectives for the next reporting period:

- Continuation of the work permitting to study the transmission of the chromosomal rearrangements.
- 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:

1. Hoffschir F., Prieur M. and Dutrillaux B.
   Diagrammatic representation for chromosomal mutagenesis studies IV. Radiation-induced rearrangements in Ateles sp. (Primate, Platyrhini).
   In press. Mutation Res.
2. Sabatier L., Al Achkar W., Hoffschir F., Luccioni C. and Dutrillaux B.
   Qualitative study of chromosomal lesions induced by neutrons and neon ions in human lymphocytes at G_0 phase.
   Mutation Research 178 : 91-97 (1987).
3. Al Achkar W., Sabatier L. and Dutrillaux B.
   Transmission of radiation-induced rearrangements through cell divisions.
   In press. Ann. Genet.

Short communications

Third workshop on heavy ions charged particules in biology and medicine. GSI Darmstadt, 07-1987.

Sabatier L., Al Achkar W., Luccioni C. and Dutrillaux B.
Chromosomal lesions induced by neon ions in human lymphocytes at Go phase.

8th International Congress of Radiation Research Edimburg 07-1987.

Sabatier L., Al Achkar W., Hoffschir F., Luccioni C. and Dutrillaux B.
Qualitative study of chromosomal lesions induced by neutrons and neon ions.

Al Achkar W., Sabatier L. and Dutrillaux B.
Transmission of radiation induced rearrangements.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D - 8042 Neuherberg

Contract no BI6-E-156-D

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

Dr. U.H. Ehling
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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 golden hamster and the mouse for the sensitivity to radiation induced mutation rate to dominant cataract alleles will be continued at 2+2 Gy.
b) The control sample size for dominant cataract mutations in the mouse will be extended.
c) The 6 Gy oocyte irradiation experiments will be continued.
d) Low dose-rate specific locus-dominant cataract mutation experiments in the mouse will be initiated.
e) Recovered dominant cataract mutations will be phenotypically and genetically characterized.

III. Progress achieved:

Procedures

Homozygous male or female (101/ElxC3H/El)F_1 hybrid mice were irradiated (0.45 Gy/min, gamma-irradiation) and mated to unirradiated partners, 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 and dominant cataract mutations. Presumed specific locus mutations were genetically confirmed by a test of alleleism at the suspected locus, while the genetic nature of presumed cataract mutations was confirmed by transmission in an outcross. The recovered dominant cataract mutations were characterized phenotypically and genetically with appropriate crosses. In a species comparison male golden hamsters or male mice were irradiated (0.45 Gy/min, gamma-irradiation, 24 h fractionation interval) and mated with unirradiated partners. Since no homozygous recessive tester stock is available for the golden hamster, offspring were screened only for dominant cataract mutations.

Results and Discussion

Due to lack of experimental results for dominant mutations, for a genetic risk estimation the sensitivity to mutation induction in oocytes has been assumed to be similar to that in spermatogonia. The first experiment to estimate the radiation-induced mutation rate to dominant alleles in mature oocytes (1-14 days after irradiation) of the mouse has been completed. Following 6 Gy irradiation 3 dominant cataract mutations (1 genetically confirmed, 1 sterile and 1 semi-lethal) were observed in 11,379 offspring. By comparison, in the same population, 18 recessive specific locus mutations (14 genetically confirmed, 1 semi-lethal and 3 not yet subjected to a confirmation test) were recovered. The observed per locus mutation rates for dominant cataract (assuming 30 loci) and recessive specific locus alleles, 0.9 and 22.6 x 10^{-5} respectively, are similar to the radiation-induced mutation rates in spermatogonia. These initial results support the assumption that the sensitivity to mutation induction in oocytes is similar to that in spermatogonia.

The comparison of the induced mutation rate to dominant cataract alleles in mouse and hamster has been continued for 2+2 Gy (24 h fractionation interval) irradiation of spermatogonia. In the mouse 2 dominant cataract and 9 specific locus mutations were recovered in 9,160 offspring. For the hamster, 1 dominant cataract mutation was observed in 5,555 offspring. The mutation in the hamster could not be confirmed due to semi-sterility and is based upon phenotypic criteria. The observed dominant cataract mutation rate in mouse and hamster, 2.2 x 10^{-4} and

1.8×10^{-4} respectively, are similar and support the assumption, required for human genetic risk estimation based upon experimental data, that the sensitivity to mutation induction in mammals is similar.

A dominant cataract mutation was recovered in a recent experiment in which DBA/2 males were treated with 3+3 Gy (24 h fractionation interval) and mated to untreated T-stock females (Favor, Neuhäuser-Klaus and Ehling, Mutation Res. 177: 161-169, 1987). The genetic nature of the lens opacity was shown in the outcross of the original F_1 female variant. Subsequent outcrosses of mutant males exclude male to male transmission and indicate X-linkage (Table). Linkage studies have shown

CONFIRMATION CROSSES

F_1 ♀ x Hybrid		F_2 ♂ x Hybrid	
	cat	normal		cat	normal
♂	15	16	♀	0	34
♀	7	16	♂	27	0

the cataract locus to be 22 cM distal to the tabby locus. This mutation represents a newly discovered gene on the mouse X-chromosome and will be useful in studying lens development and X-chromosome inactivation. X-linked cataract has been documented in humans, and the mouse mutation may serve as an animal model of a human genetic disease.

The penetrance, fertility and viability of twenty autosomal dominant cataract mutations detected among the offspring of male mice irradiated with gamma- or X-rays were studied by extensive breeding. Phenotypically the mutations are comparable in humans to juvenile hereditary cataracts with serious impairment of vision or blindness. Fourteen mutations were classified as mutations with complete penetrance without effect on viability or fertility of heterozygotes. Four other mutations were shown to be fully penetrant but the viability of the heterozygotes was impaired. Two mutations had reduced penetrance with no viability or fertility effects. Of the fourteen mutations with complete penetrance and normal viability and fertility six were shown to be homozygous viable, one semilethal and seven lethal. Penetrance, viability and fertility data are needed in evaluating the consequences of newly induced mutations in subsequent generations. The expected persistence of a mutation in a population is the reciprocol of the reduction in the probability of transmission of the mutation due to fertility or viability effects. The expected total number of individuals affected in subsequent generations is a mutation's persistence times penetrance.

IV Objectives for the next reporting period

a) The species comparison between golden hamster and the mouse for the sensitivity to 2+2 Gy irradiation 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 experiments will be initiated.

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.: Quantifizierung des strahlengenetischen Risikos. Strahlentherapie und Onkologie 163, 283-291 (1987)

Favor, J., Neuhäuser-Klaus, A., Ehling, U.H.: Radiation-induced forward and reverse specific locus mutations and dominant cataract mutations in treated strain BALB/c and DBA/2 male mice. Mutation Research 177, 161-169 (1987)

Title of the project no.: 2

Biochemical analysis of cataract mutants in mice

Head(s) of project:

J. Graw

Scientific staff:

P.M. Gopinath
A. Liebstein
T. Werner

I. Objectives of the project:

a) 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 reduced as compared to the wildtype. The cause for the reduced amount of these proteins will be investigated.
b) 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).

II. Objectives for the reporting period:

a) Characterization of gamma crystallin specific clones from murine genomic DNA-libraries (wildtype and homozygous cataract mutant Nop).
b) Continuation of the biochemical analysis of some cataract mutants recovered in radiation experiments.

III. Progress achieved:

a) Nop-cataract

Methodology
Genomic DNA-libraries of wildtype (101/ElxC3H/El)F_1 and homozygous Nop mice were established. Clones containing gamma crystallin genes were isolated by screening the libraries with cDNA probes coding for gamma-1 and gamma-2 crystallin. Restriction patterns were determined using synthetic oligonucleotides (each specific for one single gamma crystallin gene) as hybridization probes after digestion of the isolated phage DNA with different restriction enzymes. Synthetic oligonucleotides, specific for the highly conserved 5' regulatory region of the gamma-2 crystallin gene, were used in mobility shift assays to detect specific DNA binding factors.

Results and Discussion

From the genomic library of the wildtype mouse 11 clones containing gamma crystallin specific sequences were isolated exhibiting 5 different restriction patterns. In hybridizations with synthetic oligonucleotides each specific for one of the four murine gamma crystallins (gamma-1 through gamma-4) one gamma-3, one gamma-4 and two different gamma-2 crystallin clones could be identified. No gamma-1 crystallin clone was detected. One clone could not be attributed to any of the described gamma crystallin genes. From the genomic library of the homozygous Nop mouse 3 different clones were isolated containing gamma crystallin specific DNA sequences. The cloned genomic sequence did not hydridize to oligonucleotides specific for gamma-2, gamma-3 or gamma-4 crystallin. To analyze the expression of the gamma crystallin genes, a mobility shift assay was employed. A Nop-specific DNA binding factor could be detected recognizing a double stranded oligonucleotide corresponding to a 5' regulatory sequence of the gamma-2 crystallin gene. This factor was either found in the cataractous lenses but not in liver or spleen, or not found in the cataractous lenses and was present in liver or spleen. Therefore, other parts of the 5' region of the gamma crystallins will be tested.

b) Other cataract mutants from radiation experiments

Methodology
Lenses of three week old mice were prepared from heterozygous or homozygous mutants and the corresponding wildtype litter mates. The protein composition was investigated by electrophoresis (PAGE). Cytoskeletal proteins were analyzed after PAGE using Western blot techniques with antibodies against actin, spectrin and vimentin. The concentration of ATP and oxidized glutathione (GSSG) in perchloric acid extracts of single

lenses was determined using the luciferin/luciferase-system or the NADPH-dependent glutathione reductase system. The activity of superoxide dismutase (SOD) was analyzed using a pulse-radiolytic system for generation of O_2^--radicals. The activity of Na^+-K^+-ATPase was measured by the release of inorganic phosphate from ATP in the presence or absence of ouabain. The activity of transglutaminase (TGase) was determined by the incorporation of putrescine into casein.

Results and Discussion

1. A spontaneous, semidominant mutation (provisionally designated Scat, suture cataract) was detected among the control group of (101/Elx C3H/El)F_1-hybrid mice and causes a suture opacity in heterozygotes and vacuolated lenses with microphthalmia in homozygotes. The mutation has complete penetrance and no effect on fertility or viability. The histological observations suggest disturbances of the osmotic balance. The lenses of Scat mutants exhibit enhanced Na^+-K^+-ATPase activity and a decreased ATP concentration. The water content of the cataractous lenses was enhanced only in the homozygous Scat mutants. An increased concentration of GSSG was observed in lenses of heterozygotes. In the homozygotes, the GSSG concentration is similar to the wildtype level, but the SOD activity is enhanced. By PAGE, additional bands from the water soluble lens proteins could be observed in the homozygous Scat mutants. From their molecular weight it is suggested that they might represent cytoskeletal proteins. Because of the histological and biochemical observations described above it is concluded that the cataract mutation affects the osmotic state of the lens.

2. The radiation-induced, dominant mutation Cat-2^t [formerly called as R-324 (Graw et al., Mutation Res. 159, 1986, 47-54)] causes a total cataract with microphthalmia and iris dysplasia. The mutation has complete penetrance and no effect on fertility or viability. The lenses of Cat-2^t mutants exhibit enhanced content of water and GSSG as well as increased activity of Na^+-K^+-ATPase. The increase of TGase activity in the lenses of homozygotes could not be demonstrated in the liver of the mutants. A decreased ATP concentration in the heterozygous cataractous lenses was observed. The SOD activity remained unchanged in the lenses. In PAGE, additional water soluble bands could be observed both in lenses of heterozygous and homozygous Cat-2^t mutants. By Western blot analysis it could be demonstrated that some of these additional proteins cross-react with antibodies to actin and vimentin indicating destroyed lens cells.

IV. Objectives for the next reporting period:

a) Gamma crystallin specific DNA will be sequenced and compared between wildtype and Nop/Nop mutants with particular emphasis to the 5'-regulatory elements. Sequence comparisons between wildtype and Nop/Nop and between other genes will be performed.

b) The biochemical analysis of other cataract mutants in mice, recovered in radiation experiments, will be extended to other parameters. The reason for the alterations concerning the osmotic state in the Scat mutants and the cytoskeletal proteins in the Cat-2^t mutants will be further investigated.

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

VI. Publications:

Graw, J.: Die Beteiligung oxidativer Prozesse bei der Kataraktentstehung. In: Elstner, E.F., Bors, W., Wilmanns, W. (Eds.): Reaktive Sauerstoffspezies in der Medizin. Springer-Verlag Berlin, Heidelberg, New York, London, Paris, Tokyo, 1987, pp. 125-138.

Graw, J., Bors, W., Favor, J., Kratochvilova, J., Löbke, A., Michel, C., Schäffer, E., Summer, K.-H.: Oxidative Prozesse in Linsen verschiedener dominanter Kataraktmutanten der Maus. In: Elstner, E.F., Bors, W., Wilmanns, W. (Eds.): Reaktive Sauerstoffspezies in der Medizin. Springer-Verlag Berlin, Heidelberg, New York, London, Paris, Tokyo, 1987, pp. 139-142.

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 as positive control in the same offspring. To characterize genetically and biochemically the recovered enzyme-activity mutants.

II. Objectives for the reporting period:

Offspring from 1.5 or 3.0 Gy irradiated (101/ElxC3H/El)F_1 males were examined for enzyme-activity mutations. The recovered mutants were genetically characterized.

III. Progress achieved:

1. Methodology

(101/ElxC3H/El)F_1 hybrid male mice were exposed to 1.5 or 3.0 Gy gamma-irradiation (^{137}Cs, 0.45 Gy/min) and each male was mated with an untreated test-stock female, homozygous for 7 recessive loci (a, b, c, d, p, s, se). Offspring were screened for recessive mutations at the 7 specific loci and a sub-group was examined for alterations of erythrocyte enzyme activity for 10 enzymes: lactate dehydrogenase, triose phosphate isomerase, malate dehydrogenase, glucosephosphate isomerase, phospho-glycerate kinase, phosphoglycerate mutase (PGAM), glyceraldehydephosphate dehydrogenase, glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase, and glutathione reductase (GR). Individuals with specific activities (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 backcrossings to inbred C3H/El mice. Heterozygous mutants were mated inter se for investigation of homozygous viability of the mutated gene. Litter size, penetrance and expressivity were determined for the mutation.

2. Results and Discussion

No enzyme-activity mutant was detected in the 1.5 Gy irradiation group. In the 3.0 Gy experimental group, 2 mutants (GR 678 with approximately 50% activity, G6PD 909 with approximately 60% activity) were observed in 2508 offspring derived from treated postspermatogonial (pg) germ cell stages and 1 mutant (PGAM 6663 with approximately 50% activity) in 7048 offspring descendent from irradiated spermatogonia.

Dose (Gy)	Germ cell stage[a]	Enzyme-activity mutants/offspring	Specific-locus mutants/offspring
0	pg + g	0 / 3 610	22 / 248 413
1.5	pg	0 / 2 405	2 / 4 433
	g	0 / 6 467	11 / 28 964
3.0	pg	2 / 2 508	12 / 6 572
	g	1 / 7 048	6 / 24 416

[a]pg: postspermatogonial cell stages; g: spermatogonia

Twelve loci controlling the 10 scored enzymes have so far been described. The per locus mutation rate was calculated to be 6.7×10^{-5} for treated pg germ cell stages and 1.2×10^{-5} for irradiated spermatogonia. A similar difference in germ cell stage sensitivity could be observed for radiation induced specific-locus mutations.

The dominant mode of inheritance of the decreased enzyme-activities in

the mutants has been demonstrated by backcrossing with C3H/El. The penetrance was calculated as the percent of mutant offspring observed compared to that expected. For PGAM 6663 penetrance was normal, while for GR 678 penetrance was reduced to 78%. For GR 678 litter size was also reduced so that further characterization is required to determine if penetrance is truely reduced. With the exception of mutant G6PD 909, the expressivities are constant.

In offspring from intercrossing heterozygotes of the mutant lines GR 678 and PGAM 6663 no animal with a third phenotype with less than 50% enzyme activity was observed. To determine if the mutants were totally dominant or if the mutations were homozygous lethal, 20 mutant offspring from each of the two mutant lines recovered in heterozygote inter se crosses were outcrossed. All have been shown to be heterozygotes and it is concluded that the two mutations, GR 678 and PGAM 6663, are homozygous lethal.

Mutant lines GR 678 and PGAM 6663:

Mutant line	Mating ♂ x ♀	Litter size ($\bar{x}$ ± SD)	Genotype			Penetrance (%)
			+/+	+/+*	+*/+*	
GR 678	(+/+) x(+/+*)	5.7 ± 1.0	88	56	-	78
	(+/+*)x(+/+*)	2.8 ± 0.3	21	26	0	-
PGAM 6663	(+/+) x(+/+*)	7.5 ± 1.1	53	42	-	99
	(+/+*)x(+/+*)	6.3 ± 0.7	30	32	0	-

For the mutant line G6PD 909 after backcrossing heterozygous females, the offspring deficient males and females are different relative to their G6PD activity: males with roughly 20% and females with approximately 60% G6PD activity were found. Subsequent mating experiments indicated X-linkage of the G6PD enzyme activity mutation. The genetic segregation data show no disadvantage for a definite genotype and no sterile mouse has been found among the tested animals. The importance of the described mouse mutant consists in its possible use as an animal model for human G6PD deficiency.

Mutant line G6PD 909:

Mating ♂ x ♀	Litter size ($\bar{x}$ ± SD)	Genotype ♂		Genotype ♀		
		-/+	-/+*	+/+	+/+*	+*/+*
(-/+) x(+/+*)	7.1 ± 1.5	61	52	75	53	-
(-/+*)x(+/+)	7.0 ± 0.7	57	-	-	48	-
(-/+*)x(+/+*)	8.0 ± 1.3	32	18	-	39	23

IV. Objectives for the next reporting period:

To investigate the effects of genetic background on the radiation-induced mutation rate in mice the screening of enzyme-activity mutants in the offspring of fractionated irradiated males will be continued. The tested dose will be 3+3 Gy (24h fractionation interval) and strains BALB/c and DBA/2 will be chosen. Approximately 5 000 offspring per strain will be examined. Thereby a comparison with the results obtained by (101/ElxC3H/El)F_1 males will be possible.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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, E.Sporeno

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 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 have allowed us to conclude that pR works in a similar way in both mammalian and 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 uvp1 and uvp2 region products of pR plasmid, the interaction between them and the possible correlation between the expression of those functions and the pR integration sites in the host genome;
2) to transform human cells with pR plasmid, solving the problems connected with the low transformation efficiency and the lack of an appropriate selection system for cells which have no selection marker

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 AcidsResearch, 7,1541, 1979)
- Low molecular weight DNA was prepared as described by B.Hirt (J.Mol.Biol. 26,265,1967)
- Metaphase spreadsfrom mouse cell lines are obtained by standard methods after cell synchronization with BudR(17hrs) followed by a 7hrs thymidine pulse. The preparations were stained by 33258 Hoechst + Giemsa or by acridine orange followed by CBG banding. In 'situ' hybridization was performed using a modification of the technique of M.E.Harper and G.F.Saunders (Chromosoma, 83,431, 1981)
- The electroporation method for transfecting mammalian cells was performed as described by G.Chu et al. (Nucleic Acids Research,15,1311,1987)

- RESULTS

We have shown that the uvp1 (rep) region of the pR plasmid encodes a function which regulates the expression of the uvp2 region in bacterial cells This region contains the muc genes that are under the negative control of lexA gene and is the region responsible for an increased rate of spontaneous mutagenesis and resistance to UV and cytotoxic agents. It is possible to hypothesize, therefore, that the uvp1 region encodes an antagonist of lexA. This regulative region has an essential role in the expression of the UV+ functions in mammalian cells too, as confirmed by the lack of UV+ phenotype in mouse cells transformed with pR plasmid mutated in the uvp1 region for Tn5 insertion(L.Marcucci et al.,1986). In pR transfected mammalian cells the espression of the uvp2 region is correlated both to the UV-protecting effect and to the sensitizing effect to bleomycin, cis-diammine-dichloroplatinum and N-methyl-N'-nitro-N-nitroso-guanidine(R.Elli et al.,1987) showing an interaction between muc gene products and the different repair pathways involved. The possibility that pR sequences are integrated in the mouse genome is supported both by indirect and direct evidence. The stable expression of UV- and 4NQO-resistance phenotype for about 80 passages in vitro suggests the presence of integrated pR sequences.The hybridization patterns of high m.w. DNA from several pR transformed mouse lines observed after Southern blotting show the simultaneous presence of integrated and free copies of pR plasmid in the same line; however integration sites vary in the different lines. The hybridization patterns of low m.w. DNA(Hirt extraction from LA-D cells, observed by dot blot, suggest the presence of free copies. Several attempts to transform bacterial cells with pR molecules present in the Hirt supernatant were unsuccessful. A possible explanation is that such extrachromosomal molecules can undergo various types of rearrangements such as deletion and point mutation with consequent loss of either the transforming ability or the antibiotic resistance pattern. To investigate the integration sites of pR DNA, its extrachromosomal state and its organization, both 'in situ' hybridization and further hybridization

pattern analyses are necessary. For this purpose,one of the cotransformed lines,the LA-D line,has been cytogenetically characterized and compared with the untransformed LTA line,with clonal line derived from LTA line and with LA-TKO line,transformed only with the tk gene. The karyotypes of the analysed lines show numeric and structural differences as well as heterochromatin variations(P.Petrinelli et al.,1987). These differences can be explained by the effect of cloning and/or by the different selective pressures of growth,except the heterochromatin variations observed for two chromosomes of the LA-D cells,that need further investigation. The 'in situ' hybridization technique has been set-up on metaphase spreads from LA-D cells using ^{3}H-labelled pR DNA as a probe. After an 18 day-autoradiographic exposure,the metaphases analysed show a low background and a mean number of 2.9 chromosomal grains per cell. The analysis of the statistical distribution of the autoradiographic grains is in progress.A prerequisite for transforming human cells for which no selectable marker is known is the utilization of an easily selectable system such as the resistance to genetycin conferred by the pSV2neo plasmid.LTA mouse cells cotransfected with pR and pSV2 neo plasmids show the same increase in 4NQO-and UV survival observed with tk gene cotransfection,demonstrating that the observed effects of pR do not depend on the selection used. The other problem is the low efficiency of transfection that poses a serious obstacle to experiments which depend on the use of specific cell lines eg. some normal and tumoral human cell lines. For this purpose we have used the electroporation technique which involves the exposure of cells to a pulsed electric field. We have already optimized the technique with respect to parameters involving the cells,the DNA and the electric field.In fact,applying this technique on LTA mouse cells we have obtained a transfection efficiency that is 10 times higher than that obtained with the calcium-phosphate precipitation technique.

DISCUSSION

The experimental data obtained in bacterial cells show that the pR uvp2 region is under the control of a gene product codified by uvp1 region. Even if a regulative function of the uvp1 region has not yet been demonstrated in eukaryotic cells,all the Tn5 insertions in this region eliminate the ability of pR to increase UV resistance in transfected mouse cells.Furthermore to investigate the regulation of pR genes expression in eukaryotic cells is necessary the knowledge of pR integration sites in the host genome. In this respect the heterochromatin variations observed in two chromosomes of the LA-D line are interesting because of the observation that the integration of exogenous DNA could occur at or near the sites of gross chromosomal rearrangements(D.M.Robins et al.,1981) or into DNA repetitive sequences(S.Kato et al.,1986).

IV. Objectives for the next reporting period:

- To characterize the pR plasmid integration sites in different transformed mouse cell lines both by 'in situ' hybridization and by Southern blot analysis, in order to ascertain the possible correlation between the pR phenotype expression and its integration sites.
- To investigate the possible regulative function of pR uvp1 region in mammalian cells, by transfection with pR mutated for insertion or deletion in this critical region involved in the UV+ phenotype.
- To evaluate the interactions between pR products and the repair pathways of human cells cotransfected with pR and pSV2neo plasmids in response to UV and radiomimetic drugs.

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

Cecilia Birago - Istituto Superiore di Sanità. Roma. Italia

Silvia Bacchetti - Mc Master University. Dept.of Pathology, Hamilton, Ontario, USA

M. Taylor - Indiana University. Dept.of Biology. Bloomington Indiana, USA

VI. Publications:

IN SCIENTIFIC JOURNALS:

-R.Elli, A.Antonelli, P.Petrinelli, R.Bosi, F.Gigliani, L.Marcucci - The pR plasmid: a tool for discriminating between DNA lesions induced by different types of cytotoxic agents in cultured mammalian cells. Mutation Res.,191,177-181,1987.

-P.Petrinelli,R.Elli,L.Marcucci,M.Proietti,M.Vinci, A.Antonelli - Cytogenetic characterization of a mouse cell line transformed by a bacterial plasmid. Cytotechnology,1,73-76,1987.

INTERNAL REPORTS:

-R.Elli et al. - Espressione del plasmide pR in cellule di mammifero.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-E-157-UK

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.J. Porteous, Mrs. Maxine Arnott, Mr. E. Thomson

I. Objectives of the project:

To identify individuals constitutionally heterozygous for deletions involving the 11p13 region associated with the development of aniridia and/or aniridia with Wilms' tumour and associated abnormalities.

To characterise spontaneous and X-ray-induced deletions in the 11p13 region in cultured human cells.

To develop from individuals, or culture-derived cells, paired panels of human/mouse hybrid cells which contain a single human chromosome 11 segregating from the remainder of the human complement.

To isolate DNA sequences specific to the various deleted regions and to define the genes and the types of spontaneous and radiation-induced mutations involved.

II. Objectives for the reporting period:

As above

III. Progress achieved:

1. Methodology

(a) Identification of individuals with sporadic aniridia with and without Wilms' tumour and collections of blood samples of cases from colleagues in Europe and USA.

(b) Characterisation of cultured human cells with respect to spontaneous and X-ray-induced deletions in chromosome 11.

(c) High resolution banding of prophase chromosomes to accurately define all 11p abnormalities.

(d) Production of permanent lympho-blastoid cell lines from patients and of human/mouse hybrid cell lines to segregate out the human chromosome 11s.

(e) Utilising chromosome mediated gene transfer to introduce fragments of the short arm of the human chromosome 11 into mouse cells using the H-ras 1 gene as a selectable marker.

(f) Utilising pulse field electrophoresis to separate out large chromosome fragments within and surrounding the 11p13 region.

(g) Isolation of new 11p DNA probes and screening DNAs from cell lines to construct a detailed map with the region containing the gene responsible for Wilms' tumour and aniridia and the sequences involved at the sites of spontaneous and X-ray-induced chromosome breakage.

2/3. Results and discussion

In the past year we have identified several new cases with sporadic aniridia and Wilms' tumour associated with a deletion in the chromosome 11p13 region and have established lymphoblastoid cell lines from these patients. Moreover, we have obtained DNA samples from the parents of the majority of our cases. Cytogenetic analysis has shown that the parents usually have karyotypically normal somatic chromosome complements so that the predisposing mutation (deletion) most probably originates in a parental germ cell. We have therefore been accumulating DNA probes that recognise polymorphic DNA sequences on chromosome 11 with the aim of identifying the parental origins of the mutated chromosomes. We have also identified a number of cases with familial Wilms' tumour and related anomalies (WAGR) and have characterised the inherited deletions with respect of a number of genes that we have mapped to the 11p13 region. One of the closest expressed genes linked to the WAGR locus is that for follicule stimulating hormone (FSHB) which we have recently shown maps outside the WAGR locus.

We had previously reported on our strategy for using chromosome mediated gene transfer (MGT) to introduce fragments of the short arm of human chromosome 11 into mouse cells by using the H-ras1 oncogene as a selectable marker. H-ras transformed cells thus provide us with an enriched source of DNA markers for the human 11p chromosome region. We have now analysed a number of our transformants and have obtained a 200 enrichment for 11p13 markers. 'Hitch-hiking' from H-ras1 with these CMGT markers has allowed us to define seven discrete, but overlapping, intervals

spanning band 11p13 and hence the WAGR locus. Two translocations, one associated with familial aniridia and the other with Potter facies and genitourinary dysplasia, have been shown to map within the smallest region of overlap as defined by the WAGR deletions, and within the region subtended by our isolated recombinants. We are currently utilising pulsed-field gel electrophoresis to construct restriction maps of the region of interest and in the process of isolating the WAGR gene(s) will aim to determine the exact site and nature of the mutations induced in this locus.

Utilising our lymphoblastoid cell lines with 11p deletions or translocations we have produced a series of somatic cell hybrids with a mouse myeloma cell line and have successfully isolated the abnormal chromosome 11s on a mouse chromosome background. The isolation of lymphoid cells containing the desired chromosome has been considerably helped by the use of three monoclonal antibodies two of which define two different cell surface antigens (MIC 11 and MIC 4) encoded by sequences on the short arm of chromosome 11 and one (MIC 8) expressed by a long arm marker. We have shown that the two short-arm encoded cell surface markers are frequently lost in cells from Wilms' tumour patients and we have been successful in using the fluorescence activated cell sorter to select cells expressing (or not expressing) the appropriate antigens.

IV. Objectives for the next reporting period:

To define DNA sequences to at least within a few kbp of the aniridia and Wilms' tumour genes. At the same time we would hope to characterise the sites and types of mutation that are present as sporadic events, as well as those that are transmitted familially, and also determine the parental origin of the transmitted mutations.

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

Dr. Claudine Junien, Paris.
Dr. G. Fakete, 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.

VI Publications:

Porteous, D.J., Bickmore, W., Christie, S., Boyd, P.A., Cranston, G., Fletcher, J.M., Gosden, J.R., Rout, D., Seawright, A., Simola, K.O.J., van Heyningen, V. and Hastie, N.D. Proc. Natl. Acad. Sci. USA 84, 5355-5359, 1987.

Seawright, A., Fletcher, J.M., Fantes, J.A., Morrison, H., Porteous, D.J., Li, S.S-L., Hastie, N.D. and van Heyningen, V. Somatic Cell Mol. Genet. (in press).

Bickmore, W., Christie, S., van Heyningen, V., Hastie, N.D. and Porteous, D.J. Nucleic Acids Res. (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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. A. Falaschi
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Dr. U. Bertazzoni
Ist. di Genetica Biochimica
ed Evoluzionistica del CNR
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.I. SCOVASSI
Dr. C. MONDELLO
Dr. R. COLOGNOLA
Dr. R. IZZO
Dr. P. LAGOMARSINI

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 the role of ADP-ribosylation of nuclear proteins in the carcinogenic process induced in rat liver. Analysis of ADP-ribosyltransferase (ADPRT) in liver of rats treated with 2-acetylaminofluorene (2-AAF, in coll. with F. Cesarone, Genova). Study of ADPRT in Fanconi's anemia (FA) cells after treatment with DNA-damaging agents (coll. with E. Moustacchi, Paris).

Genetic analysis in UV^S CHO mutants characterized by different degrees of DNA repair defects. Study of genetic instability in "cancer family" subjects. DNA repair studies and genetic analysis in patients affected by trichothiodystrophy (TTD) with and without photosensitivity.

III. Progress achieved:

Methodology

ADPRT activity in extracts prepared from rat liver or human cells was analysed by the activity gel method. The immunological reactivity of the enzyme polypeptides was investigated by using the western blot technique. Rat liver carcinogenesis was induced by treating rats with 2-AAF according to a discontinuous feeding regimen protocol. Extent of damage to rat liver DNA was measured according to the alkaline elution technique.

The frequency of chromosomal aberrations and of sister chromatid exchanges (SCE), the expression of fragile sites 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 DNA replication rate. Genetic analysis was performed by measuring the UDS in the heterokaryons or the survival after UV light in the hybrids.

Results

The exposure of rats to a feeding regimen containing 2-AAF causes an accumulation of lesions and a progressive impairment in DNA repair capacity. Rats were treated with 0.05% 2-AAF for 3 weeks; after 1 week of recovery, the cycle (3+1 weeks) was repeated 3 times, following an experimental model of Teebor and Becker. The activity and the structure of ADPRT were analyzed using the activity gel and western blot techniques. The catalytic band of 116 kDa, present in control rats, was undetectable after 1 cycle of treatment with 2-AAF, returning progressively to a normal level within the last 2 cycles. When the aminothiol N-Acetylcysteine (NAC) was added to the 2-AAF diet, the loss of ADPRT was observed after the 2nd cycle, indicating a protective effect of the thiol agent on DNA. The extent of DNA damage and repair, measured by the alkaline elution technique, showed that the number of alkali-labile sites in DNA is significantly enhanced after the first cycle and remains high during following cycles.

ADPRT activity has been investigated in fibroblasts and lymphoblastoid cells from FA patients belonging to complementation groups (c.g.) A and B. This disease is characterized, at cellular level, by a reduced repair capacity after cross-linking agents treatment, and an impaired NAD metabolism. The results obtained indicate that the enzyme in FA cells presents the same characteristics as in control cells. The response of F.A. cells to mitomycin C (MMC) treatment is not different from that observed in normal cells.

Different degrees of alterations in cellular parameters which are indexes of genetic damage were observed in four UV^S CHO mutants. Genetic

analysis of the defect conferring UV sensitivity indicated that three mutants belong to c.g. 2 (classification of Thompson). The fourth mutant analyzed shows complementation after fusion with cells representative of the six UV^S Chinese hamster c.g. so far identified suggesting the existence of a new mutation.

Cytogenetic instability expressed as spontaneous chromosome damage was observed in lymphocytes of "cancer family" subjects; the other cellular parameters analyzed (SCE frequency, response to mutagens, expression of fragile sites) were in the normal range.

DNA repair studies in TTD patients with and without photosensitivity have been continued. Cells from photosensitive patients are unable to performe UDS and are carrying the same mutation present in xeroderma pigmentosum (XP) complementation group D. In contrast, normal level of UDS is observed in cells from TTD patients showing normal photosensitivity. Complementation analysis in heterokaryons obtained by fusion of repair-proficient with repair-deficient TTD cells, demonstrated that cells from patients showing normal photosensitivity are able to restore UDS in UV^S TTD cells.

Discussion

The relationship between DNA repair and carcinogenesis was studied in liver of rats exposed to the carcinogen 2-AAF by measuring the extent of DNA damage and the activity of ADPRT during four cycles of treatment. From the activity gel and immunoblot analysis it appears that ADPRT activity is depleted after one cycle of 2-AAF treatment, possibly as a result of an inhibition of ADPRT de novo synthesis.

The molecular basis of the reduction of repair capacity in Fanconi's anemia is not well understood. Since it has been noted in these cells a defective NAD metabolism following DNA damage, we have analyzed ADPRT activity in the two FA c.g. so far identified. We have not observed a reduction in the level of ADPRT in FA cells compared to normal cells: the molecular mass, the immunological properties and the response of ADPRT to MMC treatment are the same as in controls. These results could indicate that the molecular defect of FA is not strictly associated to the activity of ADPRT.

The assignement to the same c.g. of three UV^S CHO mutants showing different degree of mutagen sensitivity, UDS and chromosomal fragility suggests that mutations in the same gene may result in different degrees of phenotypic alterations.

The results of cellular DNA repair studies in TTD patients with and without photosensitivity indicate that the mutation determining TTD is independent of the complex set of XP mutations.

IV. Objectives for the next reporting period:

The expression of mRNA for ADPRT will be analyzed by using a cDNA probe (in coll. with H. Suzuki) in regenerating rat liver, in liver from rats treated with 2-AAF, during the HeLa cell cycle, in FA cells and human lymphocytes.

In order to localize on human genome the gene defective in hamster UV^s c.g. 7, hybrids between mutant cells and human lymphocytes will be isolated and characterized for human chromosome content and UV sensitivity. The cellular and genetic analysis of mutagen-sensitive human and hamster cells will be continued. The presence of DNA repair defects in patients showing photosensitivity and/or tumour proneness will be studied.

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

1) Sarasin A. and Mezzina M., Institut de Recherches Scientifiques sur le Cancer, B.P. 8, 94802 Villejuif (France).

2) Cesarone C.F., Istituto di Fisiologia Generale, Università di Genova, Genova (Italy).

3) Moustacchi E., Institut Curie, 26 Rue d'Ulm, 75231 Paris (France).

VI. Publications:

Publications in Scientific Journals

1) Scovassi A.I., Stefanini M., Lagomarsini P., Izzo R. and Bertazzoni U. (1987) Response of mammalian ADPRT to lymphocyte stimulation, mutagen treatment and cell cycling. Carcinogenesis 8: 1295-1300.

2) Casoli C., Tremolada F., Lori F., Scovassi A.I., Bertazzoni U., Starcich R. and Alberti A. (1987) Reverse transcriptase activity in post-transfusion non-A, non-B hepatitis: I. Characterisation and association with retrovirus-like particles in serum. Serodiagnosis and immunotherapy 1: 00-00.

3) Mezzina M., Rossignol M., Philippe M., Izzo R., Bertazzoni U., and Sarasin A. (1987) Mammalian DNA ligase: structure and function in rat liver tissues. Eur. J. Biochem. 162: 325-332.

4) Larizza L., Doneda L., Stefanini M., Francone G., Gualandri V. and Fuhrman Conti A.M (1987) Liability to chromosome damage in lymphocytes of "Cancer family" subjects: a study of spontaneous and induced chromosomal fragility. Int. Journal of Biological Markers 2: 9-17.

5) Stefanini M., Lagomarsini P., Giorgi R. and Nuzzo F. (1987) Complementation studies in cells from patients affected by trichothiodystrophy with normal or enhanced UV-photosensitivity. Mutat. Res. 191: 117-119.

6) Stefanini M., Mondello C., Tessera M.L., Botta E. and Nuzzo F. (1987) Cellular and genetic studies in three UV-sensitive Chinese hamster mutants. Cytotechnology 1: 91-94.

Short Communications

1) Scovassi A.I., Stefanini M., Izzo R., Lagomarsini P. and Bertazzoni U. - Activation of human ADPRT by alkylating and crosslinking agents and during prolonged lymphocyte stimulation. DNA repair workshop: "Molecular aspects of DNA repair". Netherlands, March 1987.

2) Cesarone C.F., Scovassi A.I., Scarabelli L., Izzo R., Orunesu M., and Bertazzoni U. - Loss of ADP-ribosyl transferase activity in liver of rats treated with 2-acetylaminofluorene. 8th International Symposium on ADP-ribosylation, abst. n. 54, Texas, May 1987.

3) Scovassi A.I., Stefanini M., Izzo R., Lagomarsini P. and Bertazzoni U. - Response of ADPRT of human lymphocytes to DNA-damaging agents and to prolonged mitogen stimulation. 8th International Symposium on ADP-ribosylation, abst. n. 114, Texas, May 1987.

4) Stefanini M., Lagomarsini P., Giorgi R., Berardesca E., Borroni G. and Nuzzo F. - Evidence of clastogenic damage in psoriatic patients under PUVA therapy. ESDR Clinical Oriented Symposium on Aging and Skin Cancer, Roma, February 1987.

5) Stefanini M., Casati A., Lagomarsini P., Giorgi R., Berardesca E. and Nuzzo F. - Chromosome instability in skin fibroblast cultures from a patient affected by xeroderma pigmentosum. International congress "DNA damage and repair", Roma, July 1987.

6) Stefanini M., Lagomarsini P., Petecca C., Bianchi C. and Nuzzo F. -DNA repair studies in a patient affected by Cockayne's syndrome. Atti Ass. Genet. Ital.34: 319 (1987).

7) Stefanini M., Mondello C., Tessera M.L., Botta E. and Nuzzo F. - Genetic complementation in UV sensitive Chinese hamster mutants. Eur. J. Cell Biol. 44 ,suppl. 21: 12 (1987).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
Abtlg.f.Biophys.Strahlenforschung
GSF
Paul-Ehrlich-Strasse 20
D - 6000 Frankfurt/Main

Telephone number 069-63033'0

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.:
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

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:

The RBE-values will be determined for the induction of DNA double-strand breaks (DSB), the production of chromosome aberrations (CA), lethality and point mutations as a function of electron energy using characteristic ultrasoft X-rays (Be_K: 120 eV; W_L: 10 keV). The formation of CA will be evaluated in different mammalian cell lines. For the induction and repair of DSB and mutation studies the eukaryote yeast will be used. With the help of synchronized cells of the mutant rad 54-3 (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. Dosimetric comparison of the ultrasoft X-ray facilities at the MRC Radiobiology Unit, Harwell, and at the GSF, Frankfurt.
2. Determination of the RBE-value of Cu_K (8 keV) characteristic X-rays for the induction of DSB.
3. Evaluation of the effectiveness of C_K (278 eV) and W_L (10 keV) X-rays to induce lethal lesions.
4. Determination of the energy and purity of the Al_K characteristic X-rays. Dosimetry of the Al_K X-ray beam.

III. Progress achieved:

1. In order to compare experimental results obtained with ultrasoft X-rays produced at the MRC/Harwell and the GSF/Frankfurt, an intercomparison of ionization chambers was performed, showing an agreement within 10% of the two chambers. Using our ionization chamber for the dosimetry of C_K X-rays in Frankfurt the survival curve of the yeast mutant rad54-3 incubated at 36°C was evaluated. Rad54-3 cells are not able to repair DNA double-strand breaks (DSB) at 36°C and are killed by one DSB/cell. The D_o-value of the exponential survival curve agreed within 6% with that determined in Harwell for the same cells. The RBE-value relative to ^{60}Co gamma rays was determined to be 2.6 as in Harwell (fig.1).

2. Based on the dosimetry presented in the annual report 1986 for Cu_K characteristic X-rays, an exponential 36°C survival curve with a D_o-value of 20 Gy was measured for rad54-3 cells. The RBE-value relative to ^{60}Co gamma rays was determined to be 0.9 (fig.1). Thus, Cu_K X-rays are as effective as ^{60}Co gamma rays to induce DSB. The high RBE-value of 2.6 of C_K X-rays for the induction of DSB suggests that electrons with energies of around 250 eV are most effective to induce DSB. Track structure calculations for electrons show that the percentage of dose imparted to matter by electrons of around 250 eV is about equal for ^{60}Co gamma rays and Cu_K X-rays which is in good agreement with the similar effectiveness of these radiations to induce DSB.

3. In order to determine the effectiveness of C_K X-rays to produce lethal lesions (misrepaired or nonreparable DSB), wild type (wt) yeast cells were kept after irradiation under liquid holding conditions for 72h before plating for the survival assay. By this treatment all reparable DSB are repaired. The results in fig.2 show that the effectiveness of C_K X-rays to produce lethal lesions is approximately the same as for 3 MeV alpha particles (L_{100}= 60 keV/µm). Because of the low dose rate (3 Gy/min) irradiations with doses higher than 300 Gy were not possible. Since the dose rate of Cu_K X-rays is even lower (0.8 Gy/min) the production of lethal lesions in wt yeast

cells was investigated using W_L X-rays (10 keV) with a track structure very similar to that of Cu_K X-rays. In the dose range of up to 700 Gy W_L X-rays are about half as effective as C_K X-rays, however twice as effective as 30 MeV electrons (not to be seen in fig.2).

4. The purity of the Al_K characteristic X-rays was investigated by determination of the energy spectrum using a calibrated X-ray proportional counter manufactured by EG&G. The spectrum shows a single bell-shaped peak with a negligible contamination of higher energetic secondary electrons from bremsstrahlung. Within the experimental error of $\Delta E/E = 0.038$, the energy of the Al_K X-rays agreed with the expected value. Using an ionization chamber especially developed for ultrasoft X-rays, the relative deviations of the dose rate $\Delta D/D$ was determined to be smaller than $\pm$ 0.01 over a circular surface of 3 cm diameter.

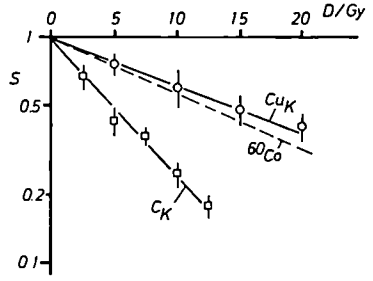

Figure 1

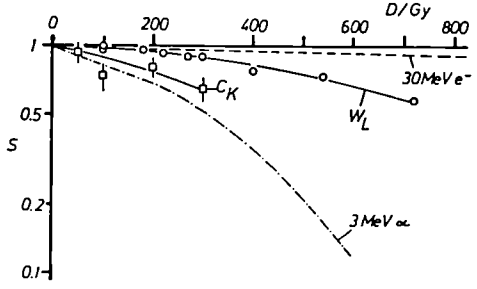

Figure 2

IV. Objectives for the next reporting period:

Dosimetry of Ti_K (4.51 keV) and F_K (0.677 keV) characteristic X-rays. Induction of DNA double-strand breaks (DSB) by Ti_K, Al_K and F_K X-rays. Investigation of lethal lesions (misrepaired or nonreparable DSB) in wild type yeast cells induced by Al_K X-rays. Investigation of point mutations induced by Al_K X-rays and ^{60}Co gamma rays as the reference radiation.

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

Prof.Dr.A.M.Kellerer, Institut für Medizinische Strahlenkunde der Universität Würzburg, FRG.

Dr.D.T.Goodhead, MRC Radiobiology Unit, Chilton, Didcot, GB.

VI. Publications:

1. Publications in scientific journals:

M.Frankenberg-Schwager, D.Frankenberg, R.Harbich: Potentially lethal damage repair is due to the difference of DNA double-strand break repair under immediate and delayed plating conditions. Radiat.Res. 111, 192-200 (1987).

D.Frankenberg: New aspect of the induction of DNA double-strand breaks by direct and indirect radiation action in yeast cells. In: Radiation Research, Vol.I, Proc.8th Int.Congr.Radiation Research, Edinburgh, July 1987, p.79.

2. Internal reports:

I.Thurn: Aufbau einer ultraweichen Röntgenstrahlenquelle und Untersuchungen im Hinblick auf eine Anwendung zur Bestrahlung biologischer Objekte, GSF-Report 14/87, Gesellschaft für Strahlen- und Umweltforschung mbH, München, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Dr. A. Goffeau
Univ. Catholique Louvain-la-Neuve
Unité FYSA, Lab. d'Enzymologie
Place Croix du Sud 1
B - 1348 Louvain-la-Neuve

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. Research aims to understand at the molecular level the mechanisms of the recombination of mt DNA and its role in repair processes.

Title of the project no.:

Head(s) of project:

Scientific staff:

F. Foury
A. Lahaye
E. Van Dyck

I. Objectives of the project:

Identification and analysis of nuclear genes involved in repair of yeast mitochondrial DNA. Relationship with DNA recombination (PIF genes) and replication (MIP genes).

II. Objectives for the reporting period:

A) Overexpression of the PIF1 gene product in E. coli and in yeast in order to purify it.
B) Identification of additional PIF genes, in order to understand their biological function.
C) Cloning of the MIP1 gene.

III. Progress achieved:

A) The nuclear gene PIF1 is required for both repair of mitochondrial DNA (mtDNA) in Saccharomyces cerevisiae (UV light, gamma-rays, ethidium bromide) and certain events of recombination involving the recognition of specific signals. This observation suggests that repair in yeast mtDNA operates through recombination. Cloning and sequencing of the gene PIF1 has shown that it encodes a poorly expressed protein (Mr = 97,500) exhibiting specific structural features of DNA helicases. Our present hypothesis is that the PIF1 protein initiates mtDNA unwinding at preferential sites, therefore facilitating stand invasion, recombination and repair. However the true enzyme function of PIF1 protein is still unknown and its determination will require its purification, which should be greatly facilitated by overproduction, and identification in an extract by an immunological test.
Firt we have overexpressed in E. coli the PIF1 gene product fused to the β-galactosidase, using the strong promoter Pr of phage lambda. The hybrid protein has been purified by SDS-PAGE gel electrophoresis and injected to a goat to obtain an antibody. Simultaneously, the PIF1 gene has been inserted in a yeast expression vector behind the strong GAL1 promoter. The overexpressed protein in yeast is recognized by the antibody. However it is toxic to the cell. Preliminary results indicate that the PIF1 gene product is membrane-bound in the mitochondria.

B) As we think that the primary biological function of the PIF1 gene is not recombination but a more vital function suc as replication or repair, we explain the absence of alteration of the mitochondrial genome in pif1 mutants (null mutants) by the existence of genes which partially fulfil the function of PIF1 gene. We made the hypothesis that it should be possible to isolate double conditional mutants (pif1 pifx) which loose their mtDNA at non permissive temperature, but are restored by a multiple copy plasmid containing the gene PIF1. After EMS mutagenesis of a pif1 null mutant, we have identified five pifx candidates. The gene PIF2 has been cloned in E. coli and in yeast.

C) We have shown by temperature inactivation curves that the nuclear mutant Mip1⁻ exhibits a highly thermosensitive mitochondrial DNA polymerase, suggesting that the mutation mip1 is located in the structural gene of the enzyme. Out of 50,000 transformants issued from three different types of yeast DNA banks, we have isolated a single type of insertion complementing the mutant mip1. However, althought the wild-type phenotype is perfectly restored in vivo by a multiple-copy plasmid, the mtDNA polymerase activity in vitro remains very low. Moreover the in vivo wild-type phenotype is not restored by a single-copy centromere plasmid. Our present conclusion is that we have cloned a phenotypic suppressor of the mip1 mutant.

Discussion

Our objectives have been reached for points A and B. We are now ready to purify the PIF1 protein, in order to determine whether it has a DNA-dependent ATPase activity and is a DNA helicase. The detection of such activities will support our hypothesis of preferential DNA unwinding elicited by the PIF1 protein at specific sites of the double helix. The isolation of new pif mutants should help to better understand the primary biological role of the PIF1 protein and its interrelation-ship with other

enzymatic complexes or molecular mechanisms (replication, repair...). Point C is not yet successful, since the gene MIP1 has not been cloned. However the study of the suppressor gene -after amplification- can be of great interest. As pointed out in the next section, we still hope to clone the gene MIP1, by transformation of yeast cells with a yeast DNA bank directly issued from yeast.

Our results support the idea that repair is not a selfish independent function in mitochondria but is closely related to fundamental functions such as replication and recombination and can only by understood in the light of these two biological processes.

IV. Objectives for the next reporting period:

A) Purification of PIF1 protein in yeast and in E. coli: solubilisation with a zwittergent, chromatography on DNA-cellulose, and other affinity columns. Detection of a DNA-dependent ATPase activity.
B) Sequence of the gene PIF2. Cloning of other PIF genes. Analysis of in vivo replication of the mtDNA in double mutants pif1 pifx. Repair properties of pifx mutants.
C) Because the gene MIP1 may not be propagated in E. coli, we will construct a yeast DNA bank without using E. coli as an intermediate, and we will use this bank to try to complement a mip1 mutant.
We will pursue the analysis of the suppressor gene complementing mip1, by performing its chromosomal disruption and its nucleotide sequence.

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

VI. Publications:

F. Foury and A. Lahaye
Cloning and sequencing of the PIF gene involved in repair and recombination of yeast mitochondrila DNA. EMBO J. (1987) 6, 1441-1449.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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, Mr. C. Carroll, Ms. A. Smith.

I. Objectives of the project:

To investigate directly the effects of radiation on the induction of chromosome abnormality in human sperm. The objectives of the project are: (1) to assess the monitoring and diagnostic contribution to radiation protection of the sperm chromosome assay (SCA); (2) if necessary, to develop improvements to the original technique to enable the SCA to be used in radiation monitoring; (3) to develop new methods of sperm chromosome analysis based on selective staining and image analysis; (4) to assess possible artefactual sources of sperm chromosome aberration induced by the SCA itself and (5) to investigate other relevant aspects of radiation exposure on sperm biochemistry and physiology.

II. Objectives for the reporting period:

(1) To complete the assessment of the SCA applied to control and radiation exposed individuals utilizing albumin and serum based culture media and extend the study to include the recently developed totally defined media. (2) To further develop the approach using defined, synthetic media into a technology that produces a maximal population of sperm capable of egg fusion. (3) To implement a new range of image analysis techniques for the monitoring of radiation-induced aberration in sperm chromosomes. (4) Determine whether repair of radiation-damaged human sperm DNA occurs in the zona-free hamster egg system. (5) Extend the range of sperm chromosome banding techniques. (6) Complete the study of the role of seminal plasma superoxide dismutase and zinc in radiation damage and repair.

III. Progress achieved:

1. Twelve radiation-exposed men were available for repeat sperm chromosome studies using the zona-free hamster egg technique. Seven were still azoospermic 1-5 y after radiotherapy. Of the remaining 5 with demonstrable sperm counts, 1 was accidentally irradiated (patient 1) and the other 4 had received radiotherapy. A summary of the results using standard techniques and using our defined synthetic media is given in Table 1. Only patients 1 and 5 yielded a reasonable number of sperm metaphases and chromosome analysis indicated that the level of aneuploidy did not differ significantly from the control group. This is in agreement with the findings of Jendeny and Rohrborn (1987) on a smaller number of metaphases but disagrees with Martin et al. (1986) whose patients tended to be exposed to lower radiation doses. No significant differences in structural rearrangements were observed although the stretching of centromeric heterochromatin particularly of chromosome 1, 9 and 16 appeared to be elevated in radiation-exposed samples.

TABLE 1	Control Group (5 normal men)	Patient				
		1	2	3	4	5
Dose (Gys)	0	0.1	35	35	35	34
% penetration standard media	35	48	0	0	0	15
% penetration defined media	73	91	0	0	22	55
Efficiency of metaphase yield standard media	12	17	0	0	0	0
Efficiency of metaphase yield defined media	26	28	0	0	2.4	16
No. of metaphases studied	1200	37	0	0	10	33
% aneuploidy	7.3	8	--	--	0	9

The major difficulty in studying the chromosomes of sperm from radiation-exposed men was caused by the poor ability of the sperm to penetrate zona-free hamster eggs and this severely limited the application of the standard technique to radiation protection studies. One cause of the poor penetration was the variable nature of some of the components of the media used for sperm penetration and gamete interaction. Fully defined, synthetic media were developed and significantly improved the penetration ability and chromosome yield of control and sub-fertile semen samples (Tomkins, Carroll and Houghton, 1988). However, it produced no improvement with the oligo- and asthenzoospermic samples regularly produced by radiation exposed men. To overcome this problem, a number of methods for the pretreatment of sperm were studied. Of these, the use of ionophore A23187 gave the highest penetration scores and metaphase yields

for control samples. However, it did not improve the performance of oligospermic samples from irradiated men.

The problems in applying sperm chromosome studies to radiation protection were eventually overcome by a major innovative advance. This involved the development of a rapid, efficient technique for the preparation of sperm samples by electropermeabilization (Tomkins and Houghton, 1988). With control samples, it resulted in sperm metaphase yields approaching 100%. More importantly, the technique could be used for oligospermic and asthenospermic samples from infertile and radiation-exposed men (Table 2). Using this technique for sperm preparation in conjunction with our defined synthetic media, we are confident that sperm chromosome analysis can now be routinely applied to radiation protection studies.

TABLE 2

	Method of sperm preparation			
	Control		Electropermeabilization	
Class of sample	Mean % penetration	Mean Sperm/egg	Mean % penetration	Mean Sperm/egg
Known fertility	35 ± 15	0.41 ± 0.1	100	8.9 ± 3.8
Oligospermia	3.0 ± 2.0	0.03 ± 0.02	57 ± 10.1	0.8 ± 0.09
Asthenospermia	15.7 ± 4.5	0.21 ± 0.05	61 ± 12.4	2.5 ± 0.9
Unexplained infertility	27.0 ± 9.7	0.32 ± 0.08	100	4.3 ± 1.9

2. Studies were carried out to establish if repair of sperm DNA could occur during the chromosome analysis procedure. Radiated sperm were introduced into hamster eggs and unscheduled DNA synthesis studied. Heterospecific repair did occur and the absence of repair contributing to gross chromosome abnormalities was unlikely. However, the level of repair was lower than might be expected on within-species evidence.

3. A new software package has been implemented for the image analysis of sperm chromosomes and has improved the identification of radiation-induced aberration in sperm chromosomes.

4. A new silver staining method has been adopted for use with R and G-banding and is useful for identifying NOR regions, satellites and fragile sites in radiation-treated sperm chromosomes. The chromomycin R method has been modified for the banding of radiation exposed sperm chromosomes.

5. A study of seminal plasma zinc and superoxide dismutase (SOD) in 43 patients and controls was completed. Mean activity of SOD following radio therapy was 3.72 Umg^{-1} protein and there was evidence of enzyme activation immediately after irradiation. SOD activity was significantly correlated with zinc levels which were correlated with sperm concentration and plasma volume. *In vitro* evidence showed that the addition of the scavangers SOD, catalase and taurine could synergistically protect sperm from UV radiation and significantly preserve their motility and egg penetrating ability in excess of 5 h after irradiation.

IV. Objectives for the next reporting period:

Previous methods of sperm chromosome analysis were unsuitable for studies of radiation-exposed men. The development of the innovative technique of pretreatment by electropermeabilization overcomes most of these problems and, with our defined media and modified fixation procedure, will enable the application of sperm chromosome analysis to radiation protection studies. In addition to regular analysis of fresh and frozen semen from radiotherapy patients, the data will be augmented by studies on sperm exposed to X, γ and UV sources in vitro.
Evaluation of the image analysis system and sequential banding techniques for radiation-induced sperm chromosome damage will be completed.
The study of media radical scavengers will be extended to include recently developed synthetic superoxide-dismutase substitutes.

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. (1987). The application of image-analysis techniques to the study of sperm chromosomes. Journal of Medical Genetics 24, 242-243.

Tomkins, P.T., Carroll, C.V. and Houghton, J.A. (1987). The application of multiple banding techniques, alone and in conjunction with image analysis, to the study of human sperm chromosomes. Heredity

Tomkins, P.T., Carroll, C.V. and Houghton, J.A. (1988). Assessment of heterospecific zona-free ovum penetration under fully defined conditions. Human Reproduction. (Accepted for publication).

Tomkins, P.T. and Houghton, J.A. (1988). The rapid induction of the acrosome reaction of human spermatozoa by electro-permeabilization. Fertility and Sterility. (In press).

Tomkins, P.T., Carroll, C.V. and Houghton, J.A. (1987). The use of image analysis techniques for the analysis of human sperm chromosomes. Proceedings of the Genetics Society of Ireland meeting, Dublin 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor.

Contract no.: BI6-E-197-D

Gesellschaft für
Schwerionenforschung
Planckstrasse 1
D - 6100 Darmstadt

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 mechanism of the action of heavy charged particles to mammalian cells.
The induction of chromosomal 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 establish a protocol of a suitable synchronization procedure
2. To investigate the changes in the cell cycle progression induced by the exposure to heavy ions
3. To measure the cell cycle dependence of radiosensitivity
4. To study chromosomal aberrations of asynchronous cell populations at different energies for various ions

III. Progress achieved:

1. Synchronization

A standard protocol for cell synchronization using centrifugal elutriation has been setup. Usually, the centrifuge is loaded with $2x10^8$ cells at room temperature and a rotor speed of 2000 rpm, starting with a flow rate of 17 ml/min. 4-5 aliquots of about 200 ml are taken at flow rates between 17 and 28 ml/min. Fig. 1 shows the main parameters of a typical run. In fig. 1a,b the number of cells per fraction and the average volume of cells in the corresponding fraction (measured with a Coulter counter) is plotted. The purity of the fractions, which is shown in fig. 1c, is measured by DNA content analysis, using flow cytometric methods. At low flow rates, excellent G_1-populations can be obtained with a purity of 95%. The maximum purity for the later cycle phases decreases to about 85% for S-cells and 70% for G_2M-cells. In future experiments, we will try to improve the purity of the G_2M-population

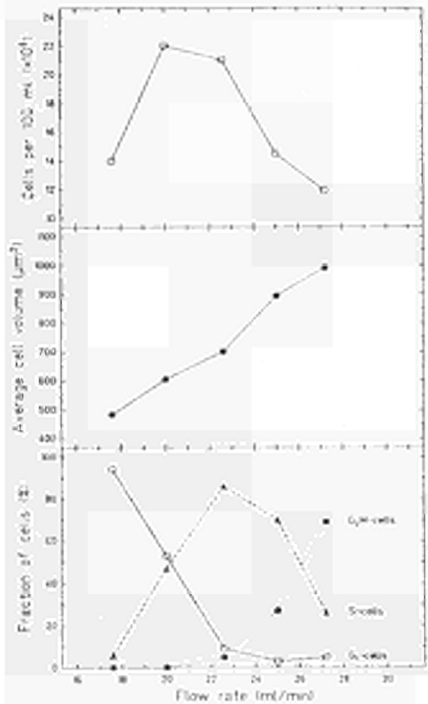

Fig. 1a

Fig. 1b

Fig. 1c

2. Cell cycle progression

Investigations on cell cycle delays induced by heavy ions have been started, because the time course of the expression of chromosomal damages is strongly influenced by these delays. The analysis of cell cycle effects has been performed by flow cytometric measurements of the DNA content of cell populations up to 24 hours after irradiation. Using asynchronous cell populations, a strong, dose dependent accumulation of cells in S-phase and in G_2M-phase was found for high energies (see fig. 2a), which is caused by a delay mainly in the late cell cycle phases. This delay also leads to a decrease of the mitotic index for several hours after exposure to the ion beam (see fig. 2b). For lower energies, the accumulation effect is less pronounced. In this case, not only a delay in the late cell cycle phases is induced, but also the G_1-cells are strongly delayed in the G_1-phase itself. This has been clearly demonstrated, using synchronized G_1-cells. Compared to the control sample, the average progression time of irradiated G_1-cells is prolonged by a factor of 2 up to at least 24 hours after exposure to a 15 MeV/u Ni-beam. This finding is in clear contrast to the effects of X-rays, where a major delay of irradiated G_1-cells can be observed only in the G_2M- region.
These experiments will be extended to heavier particle beams and higher energies in order to extract a clear relation between the parameters of the particle beam and the distortion of the cell cycle progression.

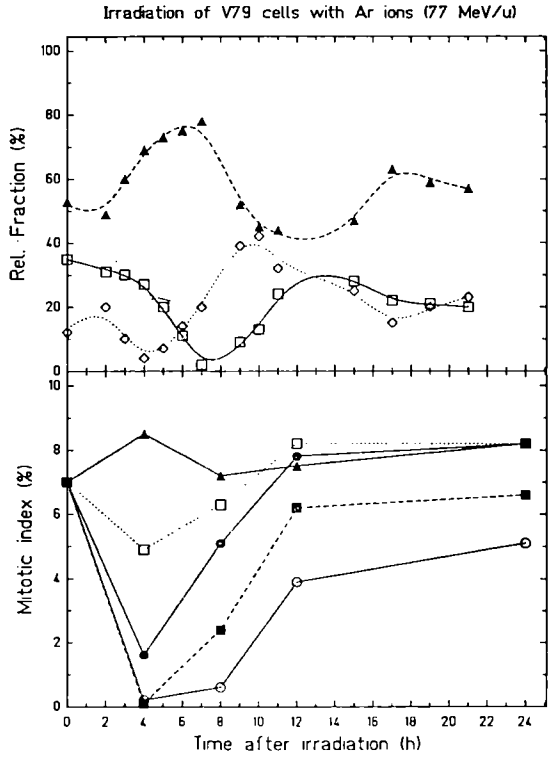

Fig. 2a

Fig. 2b

3. Cell cycle dependent radiosensitivity

Several experiments have been performed for measurements of the cycle dependent radiosensitivity of cells. For these experiments, cells synchronized in different cycle stages were irradiated with Ar, Ge and U ions and with X-rays for comparison. In general, the influence of the different cell cycle stages to the radiosensitivity is averaged when using particle radiation compared to X-rays. The particle results point to a slightly higher sensitivity of cells in the late cycle phases, if cells were irradiated in supension (see fig. 3), whereas attached cells did not show a significant cell cycle dependence of radiosensitivity.

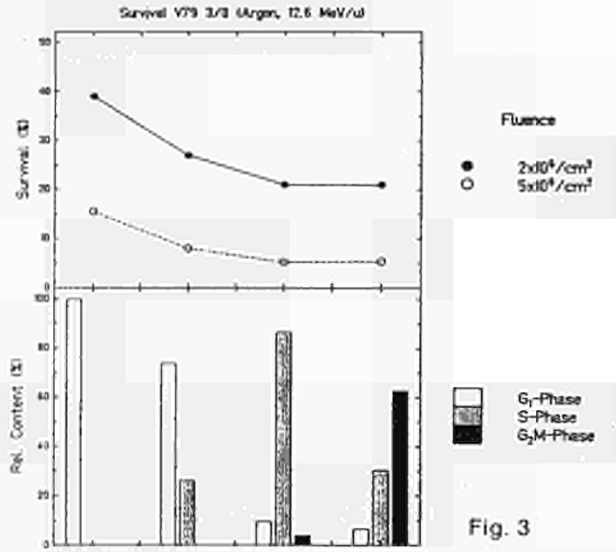

Fig. 3

4. Chromosome aberrations

Asynchronously growing V79 cells have been exposed to Ar, Ne, C and O ion beams at the UNILAC, Darmstadt and the GANIL, Caen (France). The relative frequency of various types of chromosomal aberrations has been measured as a function of time after irradiation and the particle parameters. The main results are:

- The distribution is clearly dominated by the formation of breaks, deletions and fragments, whereas exchanges are found to a lower extent.
- With increasing particle fluence the number of aberrations per metaphase increases.
- Complete and partial disintegrations of chromosomes are observed, if cells are exposed in or just before mitosis.
- The yield of abnormal metaphases depends strongly on the harvesting time of cells after irradiation. A maximum of aberrations is found 8-12 hours after exposure to heavy ions.

These experiments will be extended to synchronized cell populations at different stages of the cell cycle.

IV. Objectives for the next reporting period:

1. The changes in cell cycle induced by the particle radiation will be measured for different ions.
2. The differences in radiosensitivity of cells in different phases will be determined for light, medium and heavy ions in order to correlate chromosomal damage to letal lesions.
3. The induction of chromosomal aberrations in synchronized V79 Chinese hamster cells will be measured.
4. Chromosome analysis using cytofluorometric methods will be started.

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

VI. Publications:

G. Kraft
Radiobiological effects of very heavy ions
Nuclear Science Applications, 1987, vol.3 1-28

G. Kraft, W. Kraft-Weyrather
Biophysical aspects of track structure
Proc. of the 8th ICRR, Edinburgh, July 1987
published in Radiation Research

B. Warczak, S. Schmidt, W. Kraft-Weyrather, G. Kraft
Automated counting of cell clones: Experimental procedure and theoretical correction
to be published in: Physics in Medicine and Biology

G. Kraft
Effects of LET, fluence and particle energy on inactivation, chromosomal aberrations and DNA strand breaks
Proc. of the NATO Advanced Study Institute
"Terrestrial Space Radiation and its Biological Effects"
Corfu, Greece, October 11-25, 1987

S. Ritter, G. Kraft
Induction of chromosome aberrations in Chinese hamster cells after heavy ion irradiation
ibid.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Dr. A. Léonard
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 as biological indicators of genetic damage in man after partial body irradiation.

Head(s) of project:
Alain Léonard

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 vitro 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:

To simulate partial body irradiations human whole blood samples were exposed to different doses of gamma irradiation and mixed with different volumes of non-irradiated before culturing.

III. Progress achieved:

1. Methodology

Human peripheral blood from one healthy male donor was either kept as control or exposed to different doses (1, 2, 4, 6 or 10 Gy) of gamma radiation by means of a gammatron cobalt-60 radiotherapy unit (Siemens) at a dose rate of 0.5 Gy/min. Before culture, the irradiated blood was mixed in different ratios (1/0, 1/1, 1/3, 1/9, 1/19, 1/49) with non-irradiated blood from the same donor. After conventional culture in Ham's medium for 42 hours, colchicine was added, the cells were harvested after 45 hours and the chromosome preparations were scored by 6 experienced observers for dicentrics, rings and acentrics (including terminal and interstitial deletions, and acentric rings). The dose effect curves were analysed by a maximum likelihood method. The deviation of the observed dicentric frequencies from the expected Poisson distribution was tested by the chi-square-test (with Yates' correction) for goodness of fit.

2. Results and discussion

The scoring of chromosome aberrations in peripheral blood lymphocytes can provide a valuable estimate of the average whole-body dose after acute accidental overexposure to relatively low LET radiation uniformly distributed over the whole body. Our data (Tables 1 and 2) suggest that after an inhomogeneous exposure a selective loss of damaged cells can lead to an underestimate of the average whole-body dose. Thus, for doses from 4 to 10 Gy, the yields of dicentrics and rings observed are far below the expected values and this effect is related to the dose administered but also to the respective proportions of irradiated and unirradiated blood. The deviation of the yield of dicentric and ring chromosomes from the Poisson distribution is significant (chi-square values) only for samples irradiated with high doses and mixed in the ratio 1/1, 1/3 or 1/9 to unirradiated blood. It is probable, therefore, that in cases of partial body irradiation with relatively low doses an estimation of the inhomogeneity of the exposure by this method requires the scoring of a very large number of cells.

The well known linear-quadratic dose dependence is valid for dicentrics in pure irradiated blood. When irradiated and non-irradiated bloods are mixed, a less pronounced linear increase is seen up to 4 or 6 Gy. In such mixtures, aberration yield decreases for doses above 6 Gy as indicated by a negative quadratic b term (Table 3).

Table 1. Distribution of dicentrics in pure and mixed blood after exposure to gamma-rays.

Irradiated / non-irradiated	Dose (Gy)	Cells scored	Total number of dicentrics	Number of dicentrics per cell 0	1	2	3	4	5	6	7	8	9	X^2-value	p-value	Coeff. of dispersion
	1	600	44	558	40	2								0.02	0.88	1.01
	2	600	140	477	107	15	1							0.44	0.80	1.02
1/0	4	209	195	77	83	37	10	2						1.08	0.78	0.87
	6	137	236	27	45	30	16	15	1	3				5.41	0.24	1.16
	10	43	146	1	7	6	11	5	6	5	2			3.40	0.50	0.97
	1	600	17	584	15	1								0.09	0.75	1.08
	2	600	48	556	41	2	1							0.15	0.69	1.13
1/1	4	324	86	266	37	15	5	1						27.5	<.001	1.57
	6	523	119	464	23	20	11	3	1	1				108.0	<.001	2.38
	10	431	62	408	5	7	5	3	2	1				24.9	<.001	3.27
	1	1112	13	1099	13									0.01	0.91	0.98
	2	1180	59	1127	48	4	1							0.45	0.51	1.18
1/3	4	1178	103	1105	50	17	5	1						7.69	0.006	1.65
	6	1168	98	1122	15	16	11	2	2					28.16	<.001	2.56
	10	1082	20	1076	1	2	0	0	3					10.70	0.0015	4.18
	1	1008	11	997	11									0.02	0.87	0.98
	2	1115	17	1103	10	1	0	0	1					1.90	0.16	2.27
1/9	4	1141	29	1119	17	3	2							1.75	0.18	1.59
	6	1088	31	1069	11	6	1	0	1					4.83	0.026	2.19
	10	1025	29	1014	4	3	2	1	0	0	0	0	1	11.66	0.001	4.48
	1	1200	5	1195	5									0.05	0.82	0.99
	2	1088	19	1073	11	4								0.58	0.55	1.40
1/19	4	1028	20	1013	10	5								1.53	0.21	1.48
	6	1185	6	1182	1	1	1							2.13	0.14	2.32
	10	937	2	935	2									0.25	0.72	0.99
	1	1117	5	1112	5									0.05	0.81	0.99
	2	1105	5	1101	3	1								0.45	0.50	1.39
1/49	4	1116	6	1113	2	0	0	1						2.05	0.15	2.99
	6	1144	3	1142	1	1								0.75	0.60	1.66
	10	1081	3	1078	3									0.76	0.77	0.99

Table 2. Frequency of dicentrics (dicentric per cell) observed after the exposure to gamma-rays in mixed blood cultures and correlation with the value obtained for pure irradiated blood.

Dose (Gy)	Dicentrics per cell 1/0	1/1	1/3	1/9	1/19	1/49
1	0.073	0.028	0.012	0.011	0.004	0.004
% observed		38.6	15.8	14.8	5.5	5.5
% expected		50	25	10	5	2
2	0.233	0.080	0.050	0.015	0.017	0.005
% observed		34.3	21.4	6.5	7.4	2.0
% expected		50	25	10	5	2
4	0.933	0.265	0.087	0.025	0.019	0.005
% observed		28.4	9.4	2.7	2.1	0.5
% expected		50	25	10	5	2
6	1.72	0.228	0.084	0.028	0.005	0.003
% observed		13.2	4.9	1.6	0.3	0.1
% expected		50	25	10	5	2
10	3.39	0.144	0.018	0.028	0.002	0.003
% observed		4.2	0.5	0.8	0.1	0.0
% expected		50	25	10	5	2

Table 3. Parameters of the dose response relationships for the production of dicentrics (dicentrics per cell) according to the linear quadratic model for the pure and mixed lymphocyte cultures.

	$a \times 10^{-2}$ Gy^{-1}	$b \times 10^{-2}$ Gy^{-2}	Prob.
Pure culture	4.66+/-2.31	3.70+/-0.64	2×10^{-3}
Mixed 1/1	6.17+/-1.52	-0.46+/-0.18	$<1\times10^{-6}$
Mixed 1/3	2.94+/-0.43	-0.28+/-0.05	3×10^{-3}
Mixed 1/9	0.76+/-0.12	-0.05+/-0.01	0.96
Mixed 1/19	0.48+/-0.20	-0.05+/-0.02	1×10^{-3}
Mixed 1/49	0.08+/-0.05	-0.01+/-0.01	0.62

IV. Objectives for the next reporting period:

The observations will be completed for patients irradiated for lung cancer and for patients treated for glioma. Some studies will be made in vitro to try to find new methods to estimate the inhomogeneity of exposure.

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, Belgium

Prof. M. Lemaire, Radiation Therapy Unit, University of Liège, B-4020 Liège, Belgium

VI. Publications:

A. Léonard, G. Decat, E.D. Léonard, A. Wambersie, J. Renard. Chromosome aberrations in patients irradiated for pelvic tumours. Strahlentherapie und Onkologie 163, 795-799, 1987.

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 (in press).

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:

To obtain blood from a panel of donors, irradiate it, prepare slides from 2-day lymphocyte cultures, encode the slides, distribute them to the participating laboratories and commence our share of the microscope analysis.

III. Progress achieved:

Methods

Blood has been taken from 10 male and 10 female healthy donors aged 20-40y. It has been irradiated acutely with 0, 5, 30 and 300 mGy of 169 keV ISO wide series X-rays. Standard two-day lymphocyte cultures have been set up and sample slides checked by fluorescence plus Giemsa staining to ensure that there is an acceptably low contamination with 2nd division metaphases. Many replicate slides were produced and encoded. Slides prepared from the material irradiated with 0 and 30 mGy were distributed to all participating laboratories. Each laboratory stained their slides with conventional Giemsa and commenced scoring for aberrations.

Results

see table.

Discussion

Until the scoring is completed by each laboratory, decoded, collated and examined statistically we shall not know the results. A decision will then need to be taken on how to proceed with the work. In particular we need to determine whether sufficient donor variability exists to require that the 5 and 300 mGy material also be scored. Karyotyping will also require some collaboration between laboratories as some of the participants are more experienced in this.

Summary of cytological observations
(500 cells were analysed per group; 250 for each examinator).

Slide No	Cells with aberrations	Type and number of structural aberrations										
		Chromatid aberrations			Chromosome aberrations						Photo identification No.	
		Gap	Break	Exchange	Gap	Fragment	Ring +	Ring -	Dicentric +	Dicentric -	AL	DR
A1	13	15	1									
A3	9	10	1									
B2	5	3	1	1								
B4	10	6				4			1		1	
C1	7	6						1				1
C3	7	3				2				2	4	2
D1	2			1		1						3
D3	0											
E1	8	5	2			1						
E3	1	1										
F2	10	4	1	1	1				3		5,7	4,5
F4	8	1	3		1	3						
G1	15	7	2	1	1				2	2	8	7-10
G3	12	7				3			1	2	10	11,12
H2	4	3				1						
H4	4	2	1			1						
I2	10	5	1		2	1			1			13
I4	8	6		1		1						
J2	6	2	1			1			2		2,3	
J4	6	2	1			2			1	1		14

(+ : with fragment; - : without fragment).

IV. Objectives for the next reporting period:

To complete scoring the 0 and 30 mGy material, collate and analyse the results. It is intended to arrange a contractors' meeting at which the data will be discussed and a decision taken on whether it is necessary to score more doses. Material is already prepared at 5 and 300 mGy. The data will be examined for the presence of excessive numbers of multiply damaged cells, containing > 1 exchange aberration, which may indicate the existence of a small sub set of extra sensitive cells. The karyotyping results if available will be examined for evidence of in vivo cloning or non-random involvement of chromosomes in exchanges.

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

Dr D.C. Lloyd and A.A. Edwards, National Radiological Protection Board, Chilton, Didcot, U.K.
Dr A. Natarajan, State Univ. of Leiden, The Netherlands
Dr G. Obe, Free Univ. of Berlin, West Germany
Dr F. Palitti, Univ. of Rome, Italy
Dr J. Tawn, Sellafield, U.K.

VI. Publications:

The results of the preceding contracts held by this group of participants have been presented at a conference on Low Dose Radiation and the Immune System, May 1987, Frankfurt : D.C. Lloyd, A.A. Edwards, A. Léonard, Gh. 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. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Contract no.: BI6-E-166-NL

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
Wassenaarseweg 72
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 repair deficient mutants from mammalian cell cultures. Via replica plating clones are to be isolated which are sensitive to DNA-damaging agents. In the first instance rodent cells will be 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 tests it will be ascertained whether they belong to different complementation groups and whether they complement with repair deficient mutants isolated by other laboratories, if these mutants become available. Also, the complementing ability with known human radiosensitive disorders (XP and AT) will be investigated.

II. Objectives for the reporting period:

1. Further isolation of repair-deficient mutants.
2. Characterization of repair-deficient cell lines already obtained in terms of survival (curves for a variety of DNA-damaging agents.
3. Experiments on the stability of repair-deficient mutants. The cells will be treated with 5-azacytidine in order to establish whether they have originated by mutation or by methylation.
4. Complementation studies via cell hybridization.

III. Progress achieved:

Methodology

The methodologies have been described extensively in the 1986 report.

Results

1. Four mutagen-sensitive cell lines have been isolated in addition to the twelve which had already been obtained (see results 1986-report). Two of them were isolated from CHO cells, one sensitive to X-rays and one sensitive to MMC. From V79-cells, obtained from the University of Sussex, two X-ray sensitive mutants were isolated.
2. Cross-sensitivities.
   The X-ray sensitive mutant xrsL-1 was also sensitive to UV and the MMC sensitive mutant was not sensitive to UV. In the previous report the isolation of two mutagen-sensitive mutations from the V79 cells obtained from Sussex was described: V15-B which is X-ray sensitive and V24-B which is EMS sensitive. V15-B is also sensitive to bleomycin, slightly sensitive to H_2O_2, 4NQO, EMS and MMS while no sensitivity was found for UV and MMC. V24-B is also sensitive to MMS and not sensitive to UV.
3. The stability of V15-B was tested. The cells were treated with 5-azacytidine (5AC) and subsequently tested for reversion of the X-ray sensitivity. No effect of 5AC on reversion was found. However, the spontaneous reversion frequency of this mutant appears to be very high (10^{-3}).
4. Complementation studies.
   Three UV sensitive mutants have been crossed with representatives of the six complementation groups which have been described by Thompson. The mutants VH_1 and CA6 both belong to the first complementation group, while VB-11 complemented all six complementation groups. Therefore VB-11 is the representative of a new 7th complementation group of UV-sensitive Chinese hamster cells. VB-15 has been crossed with xrs-5 cells, isolated by Jeggo, and found to belong to the same complementation group. Crosses of our other X-ray sensitive mutants (VG-8, VC-4 and VE-5) with VB-15 or xrs-5 revealed that all three belong to a new complementation group.

Discussion

As a large number of repair-deficient mutants have now been obtained from Chinese hamster cells, there will be no emphasis on the isolation of many more mutants from these cells. As it would be worthwhile to isolate repair-deficient mutants from a human cell line a methodology for the isolation of such cell lines should be developed. The studies on complementation have revealed so far two new complementation groups, one for UV and one for X-ray and continuation of these experiments is required. For the next reporting period most effort will be directed towards the biochemical and genetic characterization of the obtained mutants.

IV. Objectives for the next reporting period:

1. Development of a methodology for the isolation of mutagen sensitive cells from a human cell line.
2. Further complementation studies via cell hybridization.

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)
Anthropogenetisch Instituut, GU Amsterdam (Dr. F. Arwert)

VI. Publications:

Zdzienicka, M.Z. and J.W.I.M. Simons. Analysis of repair processes by the determination of the induction of cell killing and mutation in two repair deficient Chinese hamster ovary cell lines. Mutation Res., 166 (1986) 59-69.

Zdzienicka, M.Z. and J.W.I.M. Simons. DNA-repair deficient mutants are induced with a high frequency in V79 Chinese hamster cells. Mutation Res., 178 (1987) 235-244.

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

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:

During the reporting period we have investigated the role of chromatin structure in UV-induced DNA repair synthesis at the level of (a) higher-order chromatin loops, and (b) defined functionally different DNA fragments. Moreover a UV-sensitive CHO mutant (VH-1) was biochemically characterized with respect to repair capacity after UV-irradiation.

III. Progress achieved:

Methodology

Experiments aimed to study the distribution of UV-induced repair events in higher-order chromatin loops have been performed with primary human fibroblasts. In order to study repair synthesis cells were grown to confluence, UV-irradiated and post-UV incubated in the presence of ^{3}H-TdR and inhibitors of replicative synthesis such as hydroxyurea (HU) and arabinosylcytosine (ara-C). The distribution of repair events in higher-order chromatin loops was investigated biochemically by digestion of DNA-nuclear matrix complexes by DNAse 1, or on single cell level using the fluorescence DNA-halo technique. Removal of UV-induced pyrimidine dimers from defined DNA fragments (single copy genes) was investigated in primary and immortalized human cells. 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 electrophoresis, Southern transfer and hybridization with specific DNA probes. The induction and removal of dimers was measured in the active adenosine deaminase gene (ADA) and the inactive 754 locus.

In the CHO mutant VH-1 repair replication was determined in cesium chloride density gradients using the standard procedures. DNA repair replication was measured over a period of 6 hrs. Accumulation of single strand breaks (SSB) in the presence of HU and ara-C after UV-irradiation was measured by alkaline elution.

Results and discussion

Eukaryotic DNA is arranged in supercoiled loops by anchorage to a protein skeleton termed nuclear matrix or scaffold. Functionally the nuclear matrix has been found to be involved in DNA replication, transcription and RNA-splicing. It is tempting to speculate, that the association of functional elements (enzymes, DNA/RNA sequences) brings about functional compartimentalization in the nucleus. We have investigated whether the repair process also occurs within the nuclear matrix compartment. Although at high UV-dose (30 J/m^2) and short pulse labelling with ^{3}H-TdR no evidence was found for association of the repair process with the nuclear matrix, a 2-4 fold enrichment of repair events in nuclear matrix-associated DNA was observed at 5 J/m^2. The preferential repair of nuclear matrix associated DNA was most prominent during the first hour after UV-irradiation (5 J/m^2). The results of chase experiments indicated, that this preferential repair occurred in DNA sequences permanently bound to the nuclear matrix. A DNA probe covering the promotor region of the ADA gene, almost exclusively hybridized to the nuclear matrix associated DNA consistent with the close association of transcriptionally active genes with the nuclear matrix reported for a variety of cell lines. Apparently the preferential repair of nuclear matrix associated DNA represents the preferential repair of transcriptionally active DNA. Two different types of UV-sensitive human cells showed an aberrant distribution of repair events in higher order chromatin loops. In xeroderma pigmentosum cells belonging to complementation group C (XP-C) preferential repair of nuclear matrix associated DNA (after 5 J/m^2) was not only observed during the first hour after UV-irradiation (as in normal human fibroblasts) but also after 18 h post-UV incubation. In Cockayne's syndrome (CS) cells the nuclear matrix associated DNA was less efficiently repaired than loop DNA. The data obtained so far are consistent with the existence of two different repair pathways: one repair system designed to repair active DNA, a second system to repair DNA sequences other than

transcriptionally active DNA (loop DNA). In XP-C the repair of active DNA is still operative, whereas in CS cells the repair of active DNA is defect. Since the overall repair capacity of XP-C and CS cells is very much reduced (15%) respectively very similar compared to normal cells, the repair of damage in chromatin exceeding the regions of active DNA, may be defective in XP-C cells, but operative in CS cells.

Direct evidence for this hypothesis has been obtained by determining the removal of pyrimidine dimers in active and inactive DNA sequences. Dimer removal was measured in 18.5 kb fragments of the ADA gene and a 14 kb fragment of the 754 locus. In confluent human fibroblasts UV-irradiated with 10 J/m², dimers are removed 2-3 fold faster from the active ADA gene than from the inactive 754 locus or the genome overall. In confluent XP-C cells, the removal of dimers from the ADA gene was very similar to normal cells, but almost absent in the 754 locus. However, in CS cells the removal of dimers from the ADA gene was slower and to much less extent than in normal fibroblasts.

Besides, in UV-sensitive human cells we have also studied the repair in the UV-sensitive cell line VH-1 originally isolated from the hamster cell line V79. DNA repair synthesis in VH-1 in response to UV was reduced to approximately 50% compared to the wild type cells. The capacity to perform the incision step of the excision repair process was analysed via the accumulation of SSB in the presence of HU and ara-C after UV exposure. In VH-1 cells the accumulation of breaks (incisions) was about half of that found in normal cells. In UV-5 cells belonging to the same complementation group, no such accumulation of breaks was observed. These data clearly indicate that two UV-sensitive mutants belonging to the same complementation group of UV-sensitive mutants, differ in repair characteristics although their sensitivity to UV is comparable.

IV. Objectives for the next reporting period:

1. The effect of specific inhibitors on preferential repair of nuclear matrix associated DNA will be investigated. We also plan to study repair synthesis at the level of higher-order chromatin loops in wild type and UV-sensitive hamster cells. 2. We will continue to study the removal of UV-induced pyrimidine in single copy genes. For this purpose interesting UV-sensitive mutants and the wild type V79 will be used as well as primary human cells hetero- or homozygote for the ADA gene. The removal of dimers in different regions of the ADA gene and in other genes (human Factor IX) will be investigated as well. 3. The establishment and characterization of a permeable cell system is part of the current research programme. 4. Biochemical characterization of UV- (VH-1) and X-ray-sensitive (V-15B, VC-4, VE-5) mutants derived from hamster V79 cells.

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

Dr. L. Mayne, Sussex Center of Medical Research, U.K.
Dr. S.T. Jacob, Pennsylvania State University, U.S.A.
Dr. R. Waters, University of Swansea, U.K.

VI. Publications:

Mullenders, L.H.F., A.A. van Zeeland and A.T. Natarajan (1987) The localization of UV-induced excision repair in the nucleus and the distribution of repair events in higher order chromatin loops in mammalian cells. J. Cell Sci., Suppl. 6, 243-262.

Kampinga, H.H., L.H.F. Mullenders and A.W.T. Konings. Effect of hyperthermia on DNA loop-size in Hela S3 cells (accepted for publication, Int. J. Radiat. Biol.).

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.

Title of the project no.: 3

DNA repair and mutagenesis

Head(s) of project: Prof. A.T. Natarajan
J.W.I.M. Simons

Scientific staff: Dr. M.Z. Zdzienicka
Drs. F. Darroudi

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:

A. Characterization of the UV-sensitive mutant VH-1.
B. Study on the fidelity in DNA-replication of GRSL mouse lymphoma cells after treatment with DNA-damaging agents.
C. Studies on the response of X-ray sensitive CHO mutants (xrs 5 and xrs 6) which are known to be defective in repair of DNA double strand breaks (DSBs) for induction of chromosomal aberrations and SCEs by different classes of chemical mutagens with known mode of action.
D. Cytogenetical characterization of transfermants of UV sensitive Chinese hamster ovary cell mutant 43-3B with amplified and non-amplified human DNA repair gene ERCC 1.

III. Progress achieved:

A. Characterization of the UV-sensitive mutant VH-1

Methodology

Reversion was studied by seeding 5 x 10^5 cells per dish (in total 3 x 10^7 cells per experiments) and irradiating the cultures from the next day onwards on 3 consecutive days with UV at a dose of 3 J/m^2 per day. Dose-response relationships have been determined for the induction of mutations at the HPRT and Na/K-ATPase loci.

UDS (Unscheduled DNA Synthesis) was determined by irradiating cells with UV followed by incubation with (^{3}H) thymidine and autoradiography. The number of grains over the nuclei of non-S-phase cells was determined.

Results

VH-1 was found to be a stable mutant as the reversion frequency was about 3.5 x 10^{-7}. None of 11 revertants had completely regained wild-type sensitivity for UV. Therefore, reversion is possibly due to suppressor mutations which restore some activity to the protein. The dose-response relationships for mutations induced by UV in VH-1 were linear. Mutation induction at the Na/K/ATPase locus was 4-fold enhanced and at the HPRT-locus 7-fold enhanced. These increases are lower than might have been expected on the basis of the approximately 10-fold enhanced UV-sensitivity with regard to survival.

VH-1 cells were compared with V79 wild type cells and with two UV-sensitive CHO mutants of complementation group 1 with respect to induction of UDS by UV. The level of UDS in VH-1 is only slightly reduced compared to wild type cells while the two other mutants of the same complementation group show only the background level of UDS.

Discussion

The data obtained with UDS (supported by data on repair replication and on incision: see project 2) indicate that a phenotypic heterogeneity exists within the first complementation group of UV-sensitive Chinese hamster mutants. It is hypothesized that the repair gene of this complementation group may have more than one functionally important domain or that the gene is involved in preferential repair of active genes.

B. Fidelity of DNA-replication in mutagenized cells

Methodology

GRSL mouse lymphoma cells were treated with ENU and the cells were seeded in subpopulations of 100 viable cells each. The cells were cultured to optimal cell density and expression of mutations, followed by the determination of the mutation frequency per culture.

Results

Five experiments have been performed with ENU, which all demonstrate a 30-50 fold enhanced mutation frequency per cell per generation in cultures which do not have a directly induced mutant. The mutation frequency appears elevated for about 10 cell generations. Three experiments have been performed with UV which point in the same direction although the degree of induced infidelity is much less. Experiments with ionizing irradiation are in progress.

Discussion

Strong evidence has been obtained for the induction of infidelity in DNA replication in mutagenized cells which occurs in addition to the targeted mutagenesis. It still remains a question whether this error prone process is due to the induction of an SOS-like response in mammalian cells. Because of the laboriousness of the experiments increase in the resolving power of the experiments is still advisable. This probably can be achieved

by raising the temperature during cell growth as this might also affect the fidelity of DNA-synthesis.

C. Studies on the response of X-ray sensitive CHO mutants

Methodology

Wild type and X-ray sensitive mutant CHO cells xrs 5 and xrs 6 (isolated and kindly provided by Dr. Jeggo) were grown in Ham's F10 medium supplemented with foetal calf serum and antibiotics. Exponentially growing cells (for G2 experiments) or cells synchronized by mitotic shake off (for G1 experiments) were treated with different concentrations of bleomycin (BL), methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), mitomycin C (MMC) and diepoxybutane (DEB) for 1 hr. For determining the frequency of SCEs, the cells were grown for 12 hr (one cell cycle) in medium containing 5 µM BrdUrd, treated for 1 hr with different chemicals, washed and allowed to recover for further period of 12 to 15 hr before fixation. Colcemid blocked metaphases after staining (Giemsa or FPG) were scored for the frequencies of aberrations and SCEs.

Results

In comparison to the wild type cells, for induction of chromosomal aberrations xrs 5 and xrs 6 were 3.0 and 4.5 fold more sensitive to BL, 2.0 and 1.5 fold more sensitive to MMS, and 3.5 and 2 fold more sensitive to EMS respectively. Both the mutants were two fold more sensitive to MMC and DEB. Similar to X-rays, G1 treatment of the mutants with BL yielded both chromosome and chromatid types of aberrations. A similar dose dependent increase in the frequency of SCEs was found in both the mutants and the wild type cells following treatment with MMS, EMS. However, with BL, there was increase in the frequency of SCEs only in xrs 6 cells.

Discussion

The increased yield of aberrations in the xrs mutants which are known to be defective in repair of DNA DSBs following treatment with alkylating agents indicates that most of the chromosomal aberrations, irrespective of the inducing agents, originate by mis-repair of DSBs. Similar to X-rays, BL induces both chromosome and chromatid types of aberrations in the xrs mutants, when treated in G1. These characteristics overlap with those found in the cells of the human readiosensitive syndrome, namely ataxia telangiectasia.

D. Cytogenetic characterization of transfermants of UV sensitive Chinese hamster ovary cell mutant

Methodology

Chinese hamster ovary cell (A-9), its UV sensitive mutant 43-3B, its transferant 43-3B (J84.1) having a few copies (about 5) of ERCC-1 gene (cloned human repair gene correcting the repair defect of 43-3B) and six amplified (about 500 copies) transfermant clones. The extent of cell survival, the frequencies of chromosomal aberrations and sister chromatid exchanges (SCEs), spontaneously as well as induced by short wave UV, 4-nitroquinoline 1-oxide, mitomycin C (MMC), methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) were determined in all the cell lines, using standard protocols.

Results and discussion

The spontaneous frequency of chromosomal aberrations in the transfermants was lower than found in 43-3B mutant cells, but still 2 to 3 fold higher than the wild type CHO cells. The spontaneous frequency of SCEs in the transfermants was less than that found in 43-3B and similar to the wild type cells (15-16 vs. 9-10 SCEs/cell). The induced frequencies of SCEs by all tested agents in transfermants is similar to that found in the CHO

wild type cells. ERCC-1 also restores the sensitivity to MMS and EMS to normal levels for cell killing and chromosomal aberrations. However, ERCC-1 could not restore completely the extent of cell killing and chromosomal aberrations induced by UV and MMC. Our results also indicate that the amplified transfermants (having multiple copies of ERCC-1 gene) restore the impaired repair function in 43-3B leading to increased sensitivity to MMC and UV better than transfermants with few copies ERCC-1 gene. The positive restoration of human repair gene in hamster cell line is of interest, because UV damage differs quantitatively between primate and rodent cells. It appears that ERCC-1 gene functions well with hamster DNA repair system.

IV. Objectives for the next reporting period:

A. Characterization of the X-ray sensitive mutants V15-B, VG-8, VC-4, VH-5.
B. Determintation of the effect of ionizing radiation on the fidelity of DNA replication.
C. Determination of the effect of temperature on the fidelity of DNA replication.
D. We shall utilize premature chromosome condensation technique to evaluate the influence of DNA DSB repair defect(s) in xrs mutants on the frequencies of radiation induced chromosomal aberrations in human lymphocytes, by fusing irradiated G_0 lymphocytes with mitotic CHO cells (wild type and 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:

Darroudi, F. and A.T. Natarajan (1987) Cytological characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. I. Induction of chromosomal aberrations by X-irradiation and its modulation with 3-aminobenzamide and caffeine. Mutation Res., 177, 133-148.
Darroudi, F. and A.T. Natarajan (1987) Cytological characterization of Chinese hamster ovary X-ray-sensitive mutant cells, xrs 5 and xrs 6. II. Induction of sister chromatid exchanges and chromosomal aberrations by X-rays and UV-irradiation and their modulation by inhibitors of poly (ADP-ribose) synthetase and -polymerase. Mutation Res., 177, 149-160.
Natarajan, A.T. Chromosomal aberrations from radiation induced DNA lesions. In: Cytogenetics (ed. T. Sharma) Oxford Univ. Press (in press).

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. Studies on (a) the effects of neutrons and X-rays on the induction of reciprocal translocations in primates; (b) factors influencing the recovery of translocations from mouse spermatogonial stem cells.
3. Studies on the induction of HPRT-mutation in lymphocytes and haemoglobin mutations in red cells.

III. Progress achieved:

1. Inducible repair in human lymphocytes

Methodology. In all these experiments, 6 h after PHA stimulation, ^{3}H-TdR (0.01 µCi/ml), tritiated water (5 µCi/ml), ^{14}C-TdR (0.01 µCi/ml) or ^{32}P (0.1 µCi/ml) was added. The cultures were X-irradiated with 50 rad 44 h thereafter (irradiation of cells in G_2 stage). Colcemid was added 1 h later and the cultures were fixed at 53 h. A number of donors were used (^{3}H-TdR: 4 donors, 3 expts; tritiated water: 1 donor, 1 expt; ^{14}C-TdR: 4 donors, 3 expts; ^{32}P: 3 donors, 3 expts) and the number of cells scored per group (control, X-rays only, radionuclide only and radionuclide + X-rays) was at least 300.

Results. Since the data are quite extensive, only the relevant comparisons are given below. In these comparisons, the column headed "expected" refers to the frequencies of chromatid breaks expected on the basis of additivity of the effects of the radionuclide and X-rays and that headed "observed" refers to the actual frequencies observed in the radionuclide + X-rays group.

^{3}H-TdR	Frequencies (%)	
	Expected	Observed
Expt 1 donor 1:	45.3	35.0
2 donor 1:	43.3	33.8
2:	37.3	32.0
3 donor 1:	40.1	32.5
Tritiated water		
Expt 1 donor 1:	39.1	36.5
^{14}C-TdR		
Expt 1 donor 1:	42.8	26.5
donor 2:	40.5	32.0
Expt 2 donor 1:	45.6	26.4
Expt 3 donor 1:	50.8	52.1

^{32}P	Frequencies (%)	
	Expected	Observed
Expt 1	52.5	35.5
Expt 2	37.6	32.0

Discussion

The results confirm and extend those presented by Olivieri et al. (Science 223: 594-597, 1984) and by us in the 1986 EURATOM report for ^{3}H-TdR in demonstrating that in the radionuclide + X-ray group, the yields of chromatid breaks are less than the sum of the yields in the "radionuclide alone" and "X-ray alone" groups. These are consistent with the hypothesis of the induction of an adaptive repair pathway. However, there are variations with respect to the amount of reduction in the frequencies of chromatid aberrations between the different expts, suggesting the existence of inter-individual variations.

2. Study on pre-meiotic germ cells of primates and rodents

Methodology

Local testicular irradiation was carried out for rhesus monkeys (Macaca mulatta) and mice. Acute 250 kV X-rays and 2.0 MeV neutrons were used. In experiments with mice pretreatments were given with the poly(ADP-ribosyl) transferase inhibitor 3-aminobenzamide (3-AB) or with hydroxyurea (HU).

After sufficient recovery of the germinal epithelium, meiotic chromosomal preparations were made and analyzed for the presence of translocations.

Results rhesus monkey

14,600 cells were analyzed from 4 monkeys receiving 0.5 Gy X-rays and 3 monkeys receiving 1 Gy of X-rays. The frequencies of translocations were 0.23% and 0.55%. An exposure of 0.25 Gy neutrons produced 0.28% translocations (3 monkeys 7200 cells).

Discussion

The X-ray data show a linear dose-effect relationship although the observed frequencies of translocations are somewhat lower than those obtained before (0.55% vs. 0.86% at 1 Gy and 0.23% vs. 0.36% at 0.5 Gy). Pooling of the neutron data with earlier results leads to an RBE of 2.1 and this is clearly lower than the value of 4 (or higher) reported for the mouse. The high cytotoxicity of neutrons for primate stem cells spermatogonia is probably responsible for this difference.

Results mouse

The data on pretreatment with HU indicated that for spermatogonial stem cells the probability that an X-ray-induced lesion in the DNA leads to cell killing is about 10 times thatfor the production of a translocation. The dose-response relationship for acute X-rays was clearly modified by 3-AB pretreatments in that (a) the initial slope is higher; (b) the position of the peak shifted to higher doses (9 Gy vs. 7 Gy), and (c) the magnitude of the peak increased (22% vs. 15%).

Discussion

The experimentally determined ratio between the probabilities that an induced lesion kills a cell or produces a translocation (the P/c ratio) of 10 for mouse spermatogonial stem cells is in good agreement with the one theoretically calculated by Leenhouts and Chadwick. The changes in the dose-effect relationship brought about by 3-AB points to a change in the P/c ratio which further stresses the radiosensitizing capacity of this compound. Theoretically also a change in the proportion of sensitive and resistant stem cells can explain the observed differences. With dose-fractionation studies it should be possible to discriminate between these two alternatives.

3. Studies on the induction of HPRT-mutation in lymphocytes and haemoglobin mutations in red cells.

Studies on somatic mutations in man
Studies with lymphocytes:

Methodology

In the previous report, we mentioned some of the major difficulties that we experienced with respect to standardization of the cloning method for detection and isolation of HPRT-mutants. We have now overcome these difficulties by adopting the methodology that is presently being used by the group of Albertini et al. in the USA. The major contribution to the success has been the addition of LAK supernatant to the culture medium as a rich source of T-lymphocyte growth factor.The LAK (Lymphokine Activated Killer cells) supernatant is in fact a 'waste product' from the LAK therapy for cancer patients.

Results

After adoption of the new methodology, HPRT mutant frequencies (MFs) have successfully been measured in 14 normal non-smoking donors and 8 smoking donors. As it has been reported in the literature that MFs are inversely correlated with the cloning efficiency (CE) and that this type of relationship was accounted for by assays with single cell CEs ≤ 0.10, it was reassuring to find the CE values in our assay were always above 0.10 (mean CE ± S.D.: 0.38 ± 0.14). For non-smoking donors of the age group 30-40 years a mean MF was calculated of 6.19×10^{-6} with 95% confidence limits of 4.53 and 8.48×10^{-6} (n=12). These MFs fit with those published by other groups of investigators. Close to 200 mutants have been isolated and frozen.At a later date a representative sample of these mutants will be analyzed at the molecular level so that the mutation spectrum in normal individuals can be established.

Studies with human erythrocytes

Methodology

As mentioned in more detail in the previous report, a collaborative effort the Depts. of Human Genetics; Cytochemistry and Cytometry; and Radiation Genetics and Chemical Mutagenesis at Leiden has resulted in the development of an image analysis technique for automated detection of hemoglobin mutants in human erythrocytes. Although several types of polyclonal antibodies have been produced, we have sofar only scored for sickle cell mutants (HbS).

Results and Discussion

Measurement of the frequency of HbS mutants in peripheral blood of non-smoking normal controls, smokers, as well as homozygotes and heterozygotes for DNA repair defects (samples received from Dr.J.Cole, Cell Mutation Unit, Brighton, U.K.) have shown large fluctuations between individuals and no significant differences between groups. If, however, the measurements within each group are pooled and if we consider all mutants detected as derived from a single, large population of stem cells, a very significant difference becomes apparent between smokers and non-smokers of the same age. These results are interpreted as follows:

- The number of multipotential blood cells in man is not larger than 10^7 and, most probably, their turnover does not involve more than 0.5% of the cell population/day.

The only conclusion possible is that, in a single individual:

- The probability of a site specific point mutation (with a frequency of 10^9) occurring in a small stem cell population is very low.
- In addition, once such a mutation has occurred, the chance that the mutated cell differentiates and appears eventually in the peripheral blood is also considerably low.

Mutations can also involve committed red cell progenitors. The target population would be , in this case, much larger, but a mutation occurring at this stage would be amplified very little and could escape detection. Considering this situation it is not surprising to observe such large fluctuations of the mutant frequency between individuals.

Objectives for the next reporting period

Lymphocytes

Because the cloning method for detection of HPRT mutants is now under control a start will be made with the detection of mutants in patients exposed to low doses of radiation. In a recent study by Seifert et al.

(Mutation Res.191,1987, 57) evidence was presented indicating that HPRT mutant frequencies were enhanced in peripheral T- lymphocytes from heart disease patients undergoing ventriculographic tests involving injection with technetium^{99m}. Because the total exposure was of the order of 10-15 mGy of gamma radiation, a significant increase of the mutant frequency is quite unexpected. Therefore, we are of the opinion that it is desirable that such an observation is checked experimentally by another group of investigators. In the past few months we have made the necessary arrangements for a collaborative study with the Departments of Nuclear Medicine and Cardiology of the Academic Hospital of the State University of Leiden.

Simultaneously, we will determine mutant frequencies in patients exposed to much higher doses of X- or gamma rays.
As an indicator for induced chromosomal damage, all donors will also be screened for the presence of micronuclei in binucleated lymphocytes.

Erythrocytes
In order to make the estimation of hemoglobin mutations more meaningful, we are planning to adopt in 1988 the following two strategies:
- We will pool the measurements of HbS mutants carried out in a homogeneous population (same age group, smoking habits and/or exposure to irradiation) of about twenty individuals.
- A mixture of antisera against several point mutations (HbS, S.José, Leiden) will be used in order to detect simultaneously two types of nucleotide substitution and a deletion. The sensitivity of this assay will be increased several fold by the prior isolation and concentration of the three classes of specific antibodies by means of affinity chromatography.

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. van, K. Hanson, H.J. Goudzwaard and A.T. Natarajan (1987) Influence of post X-irradiation treatment with Neurospora endonuclease on the symmetry of chromatid interchanges in CHO cells in vitro. Mutation Res., 190, 145-147.

Buul, P.P.W. van, A. Tuinenburg-Bol Raap, A.G. Searle and A.T. Natarajan (1987) A search for radiosensitive mouse mutants by use of the bone-marrow micronucleus technique. Mutation Res., 191, 163-169.

Buul, P.P.W. van (1987) Schatting van genetische stralingsrisico's voor de mens. Vakblad voor Biologen, 67, 199-203.

Buul, P.P.W. van, H. Wiersema and J.F. Richardson Jr. (1987) The induction of chromosomal aberrations by ionizing radiation in macaque spermatogonia: effects of low dose rates and different species. Abstract, Int. J. Radiation Biol., 51, 745.

Tates, A.D. en P.P.W. van Buul (1987) Genetische effecten ten gevolge van blootstelling aan ioniserende straling. In: Grondbeginselen stralingsfysica en radiobiologie voor medische toepassingen (eds. J.J. Broerse en mw.) Rijswijk RBI-TNO Leiden IRS ISBN90-70639-08-4 pag. 368-405.

Tates, A.D., L.F. Bernini, A.T. Natarajan, J.S. Ploem, J. Cole, M.H.L. Green and C.F. Arlett (1987) Detection of somatic mutants in erythrocytes and lymphocytes of normal, exposed and repair-deficient humans. XVII Annual Meeting of the European Environmental Mutagen Society, Zürich (abstract and symposium paper).

Verwoerd, N.P., L.F. Bernini, J. Bonnet, H.J. Tanke, A.T. Natarajan, A.D.

Tates, F.H. Sobels en J.S. Ploem (1986) Somatic cell mutations in humans detected by image analysis of immunofluorescently stained erythrocytes. Proc. International Symposium on Clinical Cytometry and Histometry, Schloss Elmau, F.R.G. (in press).

Tates, A.D., L.F. Bernini, A.T. Natarjan, J.S. Ploem, J. Cole, F. Richmond, M.H.L. Green and C.F. Arlett (1987/88) Detection of somatic mutants in man: HPRT mutations in lymphocytes and hemoglobin mutations in erythrocytes. Mutation Res. (in preparation).

Abstract

Tates, A.D., L.F. Bernini, A.T. Natarajan, J.S. Ploem, J. Cole, M.H.L. Green and C.F. Arlett (1987) Detection of somatic mutants in erythrocytes and lymphocytes of normal, exposed and repair-deficient humans. XVII Annual Meeting of the European Environmental Mutagen Society, Zürich (abstract and symposium paper).

Title of the project no.:

5

Studies on mutations and their repair in Drosophila

Head(s) of project: Prof. Dr. P.H.M. Lohman

Scientific staff: Dr. J.C.J. Eeken, Dr. W. Ferro,
Dr. A. Pastink, Dr. A.P. Schalet

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 and (3) to study the influence of mobile elements and transposition events on specific radiation induced damage.

II. Objectives for the reporting period:

1. The comparison of the repair capacity of the repair-deficient cell lines mei-9 and mus-201 with a repair-proficient control using nucleoid sedimentation.
2. The genetic effects of repair-deficient mutants in somatic cells.
3. Isolation of radiation-induced white mutations in a repair-deficient background.
4. Sequence analysis of ENU induced vermilion mutations recovered in a repair-proficient background.
5. Molecular analysis of the complete P-elements of two MR strains.
6. The effect of the amount of middle repetitive sequences on the spontaneous mutation rate.

III. Progress achieved:

1. Repair capacity of repair-deficient mutants.

Nucleoid sedimentation experiments were performed to characterize the repair capacity for strand breaks of two permanent cell lines deficient in excision repair (mei-9, mus-201). After UV-treatment (10 J/m²; 256 nm.) the number of breaks detected in the mei-9 and mus-201 cells is lower than in the repair-proficient (control) cells. This indicates that the first step of the excision-repair process, the incision, is impaired in these mutants. The repair of X-ray-induced (10 Gy) breaks, as measured by nucleoid sedimentation, appears normal in the mutants mei-9 and mus-201. The biochemical characterization of these mutants will be extended to include the question of preferential repair at the level of individual genes (active genes versus inactive genes).

2. The genetic effects of repair-deficient mutants in somatic cells of the intact organism.

Since the biochemical characterization of repair-deficient mutants is performed in, basically, somatic cells and the genetic effects of these mutants is determined in cells of the germline, we decided to investigate the effect of the repair-deficient mutants in a conventional in vivo somatic mutation test. Spots of mutant tissue can be determined in an otherwise wild-type eye or wing. These spots of mutant tissue can arise from mutations and deletions of the marker genes used, as well as from somatic recombination between homologous chromosomes, carrying the markers. Two such systems are well defined. The first uses morphological markers localised on the X-chromosome and detectable in the eye (w/w^{co}-system), the second system has autosomal markers detectable in the wing (mwh/flr-system). Stocks are constructed that carry these markers in combination with any one of the repair-deficient mutants mei-9, mei-41 or mus-101. Pilot studies have been performed with these new strains and it can be concluded sofar that (1) the excision repair-deficient mutant mei-9 has a similar effect on spontaneous mutational events as can be determined in germ-cells, (2) the post-replication repair-deficient mutants mei-41 and mus-101 show smaller effects on spontaneous events than mei-9. These experiments will be continued measuring the effects after X-irradiation.

3. Isolation of radiation-induced white mutations in a repair-deficient background.

To investigate whether repair-deficient mutants may affect the molecular nature of the recovered induced mutations, we isolated 12 whole body white mutants after crossing irradiated (15 Gy) normal males to females, deficient in excision repair (mus-201). The specific mutation frequency at the white locus, detected in this experiment (12/52.741) is not different from that determined earlier using repair-proficient females. However, the frequency with which mosaic mutations were recovered is 2-3 times higher if repair-deficient females are used as compared to repair-proficient females. This indicates that at least part of the X-ray induced damage doesnot inhibit DNA-replication. From the 12 isolated whole body mutations, 4 are multi-locus deletions and 1 is a translocation. The remaining 7 mutations appear 'normal' according to Southern-blot hybridizations of restriction enzyme patterns. We intend to clone and analyse a number of the remaining 7 mutations in order to determine the exact nature of these mutations.

4. Sequence analysis of ENU induced vermilion mutations recovered in a repair-proficient background.

In case of a spectrum-analysis of chemically induced mutations, the white locus is less suitable. Chemically induced lesions will be, predominantly, base-changes. The coding region of the white locus is however 2600 bp long. To analyse chemically induced mutations, we will use vermilion mutations. The coding region of this gene is only 1200 bp long, and it is situated on a fragment that can be cloned easily. Many ENU induced vermilion mutations are isolated in our laboratory by the group of Prof. Dr. E.W. Vogel. A number of these mutations have been analysed. The predominant change induced by ENU appears to be a transition of GC to AT.

5. Molecular analysis of the complete P-elements of two MR strains.

MR-strains are characterized by increased spontaneous mutation frequencies (by a factor of 5-10) in particular crosses ('hybrid-dysgenesis'). This increase is due to an activated transposition process involving one particular family of mobile elements, the P-element. This family consists of homologous elements of varying size, all however preserve a 31 bp inverted repeat at the ends. The driving force behind the transposition of the members of this family is a 3 kb complete P-element. We have analysed at the molecular level the organization of complete P-elements isolated from two MR-strains, MR-h12 and MR-T007. The difference between these two strains is that, as measured by the capacity to excise smaller, defective P-element members of the family from the genome, MR-h12 is weaker (by 5 times) than MR-T007. From MR-h12 we have isolated one complete P-element most probably responsible for the excision effect and five from MR-T007. At the molecular level all except one (from MR-T007) appear normal with respect to their size as well as the 31 bp inverted repeat and the 8 bp direct duplication that arises upon integration of these elements. We will select two of these complete elements and determine whether individual genetic differences can be detected.

6. The effect of the amount of middle repetitive sequences on the spontaneous mutation rate.

The purpose of this investigation was to determine whether there exists a correlation between the spontaneous mutation rate and the amount of dispersed middle repetitive (mobile) DNA sequences. The amount of these sequences is seven times higher in Drosophila melanogaster than in Drosophila simulans. Therefore, if a correlation exists, the spontaneous mutation rate in D. melanogaster should be seven times higher than that in D. simulans. We isolated an X-ray induced X-chromosome inversion, that reduces the crossingover between white and forked, two X-linked visible markers, to less than 1%. This inversion was subsequently used to determine the sex-linked recessive lethal mutation rate in D. simulans males of a laboratory strain marked with white. The frequency of these lethals found is not different from that observed in D. melanogaster.

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 genes in repair-proficient and repair-deficient cell lines (mei-9 and mus-201). (2) The genetic effect of repair deficient mutations (mei-9, mei-41 and mus-101) on somatic tissues. (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 deficienties in heterozygous condition.

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.W.M. de Jong and M.M. Green (1987) The spontaneous mutation rate in Drosophila simulans, Mutation Res. 192, 259-263.

Eeken, J.C.J., R.J. Romeyn, A.W.M. de Jong and A. Pastink (1987) The genetic and molecular analysis of MR-strains (P-strains). In "Tenth European Drosophila Conference" pp 97 (abstract).

Pastink, A. (1987) Moleculaire analyse van geinduceerde mutaties in D. melanogaster. Bijeenkomst Sectie voor Genetische Toxicologie van de Nederlandse Vereniging voor Toxicologie, okt. 13, Bilthoven (abstract)

Pastink, A. and H. Vrieling (1987) Molecular analysis of mutations in endogenous genes in cultured mammalian cells and in Drosophila melanogaster. XVII Annual Meeting EEMS, july 19-23, Zurich (abstract)

Pastink, A., A.P. Schalet, C. Vreeken, E. Paradi and J.C.J. Eeken (1987) The nature of radiation induced mutations at the white locus of Drosophila melanogaster, Mutation Res. 177, 101-115.

Pastink, A., C. Vreeken, A.P. Schalet and J.C.J. Eeken (1987) Radiation induced deletion formation at the white locus of D. melanogaster, Abstract DNA Repair Workshop "Molecular Aspects of DNA Repair", Noordwijkerhout, March 1-5, 1987 (Poster abstract).

Schalet, A.P. (1986) The distribution of and complementation relationships between spontaneous X-linked recessive lethal mutations recovered from crossing long-term laboratory stocks of Drosophila melanogaster. Mutation Res., 163, 115-144.

Sobels, F.H. and J.C.J. Eeken (1987) Mutation by transposition of P-elements in Drosophila and genetic risks, Biol. Zent.bl. 106,

129-139.
Vegt, G.B. and W. Ferro (1987) Studies on mutagen-sensitive strains of Drosophila melanogaster. X. Repair of radiation-induced DNA damage in primary cell cultures after irradiation with X-rays. Mutation Res. 177, 95-100.
Vrieling, H. and A. Pastink (1987) Molecular analysis of radiation-induced mutations in cultured mammalian cells and Drosophila melanogaster. Int. J. Radiat. Biol., 52, 643 (abstract).

Title of the project no.: 6

The production of chromosome aberrations in human lymphocytes by low doses of X-rays

Head(s) of project: A.T. Natarajan

Scientific staff: K. Sankaranarayanan
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:

To obtain blood from a panel of donors, irradiate it, prepare slides from 2-day lymphocyte cultures, encode the slides, distribute them to the participating laboratories and commence our share of the microscope analysis.

III. Progress achieved:

Methods

Blood has been taken from 10 male and 10 female healthy donors aged 20-40y. It has been irradiated acutely with 0, 5, 30, and 300 mGy of 169 keV ISO wide series X-rays. Standard two-day lymphocyte cultures have been set up and sample slides checked by fluorescence plus Giemsa staining to ensure that there is an acceptably low contamination with 2nd division metaphases. Many replicate slides were produced and encoded. Slides prepared from the material irradiated with 0 and 30 mGy were distributed to all participating laboratories. Each laboratory stained their slides with conventional Giemsa and commenced scoring for aberrations.

Results

The cultures were shown to contain 95% or more first division metaphases. It is intended that each laboratory shall score 500 metaphases per donor per dose. All cells containing 1 or more exchange aberration are being photographed for karyotyping and the x,y stage co-ordinates recorded so that the cells can be re-examined if required.

In the NRPB laboratory approximately 90% of our share of the scoring of the zero and 30 mGy material had been completed by the end of 1987.

Discussion

Until the scoring is completed by each laboratory, decoded, collated and examined statistically we shall not know the results. A decision will then need to be taken on how to proceed with the work. In particular we need to determine whether sufficient donor variability exists to require that the 5 and 300 mGy material also be scored. Karyotyping will also require some collaboration between laboratories as some of the participants are more experienced in this.

IV. Objectives for the next reporting period:

To complete scoring the 0 and 30 mGy material, collate and analyse the results. It is intended to arrange a contractors' meeting at which the data will be discussed and a decision taken on whether it is necessary to score more doses. Material is already prepared at 5 and 300 mGy. The data will be examined for the presence of excessive numbers of multiply damaged cells, containing >1 exchange aberration, which may indicate the existence of a small sub set of extra sensitive cells. The karyotyping results if available will be examined for evidence of *in vivo* cloning or non-random involvement of chromosomes in exchanges.

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

1. National Radiological Protection Board, Chilton, U.K. (Dr. D. Lloyd)
2. Free University of Berlin, Germany (Prof. G. Obe)
3. CEN/SCK Mol, Belgium (Dr. A. Léonard)
4. BNFL, Sellafield, U.K. (Dr. J. Tawn)
5. University of Rome, Italy (Dr. F. Palitti)

VI. Publications:

The results of the preceding contracts held by this group of participants have been presented at a conference on Low Dose Radiation and the Immune System, May 1987, Frankfurt: D.C. Lloyd, A.A. Edwards, A. Léonard, Gh. 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. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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: 071-148333-6175

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. P.H.M. Lohman

Scientific staff:

Prof. 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 estimates;
2. To make use of these data and those that bear on the severity of these diseases and arrive at estimates of detriment;
3. To make an in-depth analysis of the mutation component of diseases of complex aetiology.

All these are carried out in close collaboration with Dr. Czeizel in Budapest and item 3 additionally, in collaboration with Professors J.F. Crow and C. Denniston of the University of Wisconsin, Madison, U.S.A.

II. Objectives for the reporting period:

The principal objective of the work carried out in 1987 has been (i) to make a detailed analysis of the prevalence of spontaneously-arising diseases of complex aetiology (but with a genetic component), in Hungary, compare these estimates with those published in the literature for other countries and summarise their epidemiological features, and (ii) to make use of these data and those on mortality and other aspects and arrive at estimates of detriment -- handicap or harm at the individual and societal levels.

III. Progress achieved:

Methodology

The disease entities selected for consideration represent a sub-set of those given in the WHO Manual on the International Classification of Diseases and Causes of Death. Base-line figures on population sizes, number of deaths and causes of mortality were extracted from the Hungarian Demographic Year Books while data on age-standardized prevalences, age ranges and mean ages at onset of these diseases, and heritability estimates were based on epidemiological studies carried out in Hungary. Data published in the literature were used to make comparisons with the Hungarian data. The indicators of detriment used were: years of life lost, potentially impaired and actually impaired. While the first two could be estimated from the epidemiological data, for the latter, we made use of the Records from the Office of Hungarian Medical Specialists which provided information on premature retirement, the indicator used herein for actual life impairment.

Results and discussion

The total Hungarian prevalence of the ± 25 diseases included in the analysis (Graves' disease, diabetes mellitus, gout, schizophrenia, affective psychoses, multiple sclerosis, epilepsy, glaucoma, essential hypertension, acute myocardial infarction, varicose veins of the lower extremities, allergic rhinitis, asthma, peptic ulcers, idiopathic proctocolitis, cholelithiasis, celiac disease, calculus of the kidney, atopic dermatitis, psoriasis, systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Scheuermann disease, and adolescent idiopathic scoliosis) is 6541 per 10^4 of the population; this figure refers to lifetime prevalence (and <u>not</u> the number of individuals per 10^4 livebirths) since several individuals may have more than one disease. While there are some individual differences, the overall total prevalence that can be estimated from other studies published in the literature (6367/10^4 of the population), is similar to that in Hungary.

The mortality data, the analysis of which is complete, suggest that with the exception of epilepsy, early mortality (i.e., death between age 0 and 19) is rarely associated with these diseases. However, mortality between the ages of 20 and 69 is considerable, being in the range from 80 to 100% for schizophrenia, affective psychoses, multiple sclerosis and systemic lupus erythematosus, 60-79% for Graves' disease and epilepsy, and 40-59% for diabetes mellitus, gout, acute mycocardial infarction, asthma etc. Diseases such as allergic rhinitis, glaucoma, atopic dermatitis and psoriasis are not principal causes of death.

The estimated annual mortality due to these diseases is about 20/10^4 of the population with acute myocardial infarction being the predominant cause (13.5/10^4). The figure of 20/10^4 is about 15% of the total mortality in Hungary due to "all causes" (listed in the WHO Manual).

In terms of the mean number of years of life lost (the difference between age-specific life expectancy and mean age at death) -- an indicator of detriment at the individual level -- epilepsy tops the list (31 yrs) followed by affective psychoses (20 yrs), multiple sclerosis (19 yrs), systemic lupus erythematosus (17) and schizophrenia (14 yrs). Diseases such as proctocolitis, acute myocardial infarction, Graves', diabetes mellitus and asthma cause between 7 to 9 yrs of life loss on the average whereas those such as cholelithiasis, essential hypertension, rheumatoid arthritis etc. cause fewer years of life loss (2-6 yrs). Finally, those such as glaucoma, gout, allergic rhinitis, celiac disease,

psoriasis, Scheuermann disease and scoliosis are not associated with any life loss.

The total number of years of life loss -- an indicator of detriment at the population level -- is about 3500 yrs/10^4 individuals. To this figure, acute myocardial infarction and affective psychoses contribute significantly (about 2100 yrs and 600 yrs, respectively). Considering that the total life time prevalence of these diseases is around 6500/10^4 individuals, the estimated total life loss is not remarkably high. This is because of the fact that diseases with high prevalences are not those associated with high mean number of years of life loss.

IV. Objectives for the next reporting period:

For the next reporting period, analyses will be focused on the mean number of years of potentially impaired life (the difference between the mean age at onset and the mean age at death) and mean number of years of actually impaired life; the latter will take into account, the mean age at premature retirement, rate of premature retirement and the actual mean duration of the illness. There are already indications that both the mean and total number of years of potentially impaired life would be substantial for these diseases and that the impact on medical facilities could be considerable. Further, if time permits, we will extend this kind of analysis to mental retardation: aetiology and public health aspects.

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.

Prof. J.F. Crow and Prof. C. Denniston, both at the Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

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

Contract no.: BI6-E-190-UK

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.: 1

Mutation and chromosome aberration in V79 cells by neutrons

Head(s) of project:

Dr A Morgan

Scientific staff:

Dr P D Holt
Dr G R Morgan
Mr C J Roberts

I. Objectives of the project:

To establish whether there is any variation of the mutation and chromsome aberration response of mammalian cells with neutron dose-rate, and in particular whether an inverse dose-rate effect occurs, such as has been reported by Hill and Elkind (EUR 8084, 1982) for the induction of transformation in C3H 10T½ mouse cells by fission neutrons. The purpose is to understand the inverse dose-rate phenomenon.

II. Objectives for the reporting period:

1. Measurement of the mutation frequencies induced in V79 cells in stationary phase by doses of 2 Gy and 0.2 Gy of the 2.1 MeV neutron spectrum at a high dose-rate.

2. Measurement of the survival of V79 cells in stationary phase exposed to a range of doses of 2.1 MeV neutrons at a low dose-rate.

3. Evaluation of the influence of dose-rate on the expression time of mutants, using ^{60}Co gamma-ray irradiation.

III. Progress achieved:

The mutation frequencies induced in V79 cells in stationary phase have been measured for doses of 2 Gy and 0.8 Gy of our 2.1 MeV neutron spectrum at both high and low dose-rates. The dose-rate was 0.1 Gy min^{-1} and the low dose-rate was such as to deliver the specified dose in 10 hours. The lower dose was 0.8 Gy instead of the 0.2 Gy as originally intended, because this was the lowest neutron dose at which a measurable mutation frequency above background could be produced. This simply reflects the spontaneous mutation frequency of mammalian cells and would not compromise the detection of an inverse dose-rate effect. The temporal expression of the mutation frequency has also been measured at each dose-rate for each dose, to ensure any inverse dose-rate effect is detected. In addition, the mutation frequency and expression time of ^{60}Co γ-ray induced mutations has been measured over a range of doses and found to be in agreement with published data from other laboratories.

The data have not yet been fully analysed, but a preliminary assessment suggested that the mutation frequency was independent of dose-rate for neutrons, while lowering the dose-rate reduced the mutation frequency for γ rays as expected.

The survival of V79 cells in stationary phase was measured for low dose-rate irradiation by a range of doses of 2.1 MeV neutrons at a dose-rate of 0.2 Gy h^{-1}. There was no difference between the survival curve obtained and that obtained previously using a high neutron dose-rate (about 0.1 Gy min^{-1}).

IV. Objectives for the next reporting period:

It is important to discover the exact circumstances in which an inverse dose-rate effect can occur. The experiments in 1987 have been carried out using stationary-phase cultures. However, the experiments in which an inverse dose-rate effect was reported for neutron-induced transformation were carried out with actively growing cells. In order to find out whether this difference is important, the effect of neutron dose-rate on survival, and on mutation frequency, will be measured using actively-growing cells. A parallel experiment will be carried out with ^{60}Co γ rays to provide a positive control.

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

VI. Publications:

1. G.R. Morgan, C.J. Roberts and P.D. Holt (1987) The influence of dose-rate on the biological efect of low doses of radiation. (Abstract) Proc. 8th International Congress of Radiation Research, Vol. 1, p.129.

2. G.R. Morgan, J. Oliver, C.J. Roberts and P.D. Holt (in preparation) The effect of dose and dose-rate on survival and chromsome aberration frequency in V79 Chinese hamster cells.

Title of the project no : 2

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 find out whether the inverse dose-rate effect reported by Hill and Elkind (EUR 8084, 1982) for neutron irradiation of C3H 10T½ mouse cells can be reproduced in this laboratory, using a range of high-LET radiations, and to investigate its mechanism.

II. Objectives for the reporting period.

1. Measurement of the transformation frequency induced by ^{238}Pu α-particles for a total dose of 0.2 Gy and at dose rates of 0.005 and 0.1 Gy min^{-1}.

2. Transformation by accelerator-generated neutrons with a spectrum similar to fission neutrons at dose-rates of about 0.1 and 0.005 Gy min^{-1}.

3. Measurements of transformation using ^{60}Co γ-rays at a high dose-rate in order to establish RBE values for the α particles and fission neutrons.

4. Transformation measurements were also planned using a 24 keV neutron beam from the PLUTO reactor; the dose-rate is approximately 0.005 Gy min^{-1}.

III. Progress achieved:

The transformation frequency per surviving cell was measured at a dose of 0.29 Gy of ^{238}Pu alpha particles, given at high and low dose-rates (1.0 and 0.05 Gy min^{-1}). Within the statistical uncertainties of the method, the results showed no difference in the transformation rate. However, both the radiation-induced and the background transformation responses were much higher than the transformation frequencies found in other laboratories. The serum used for this work was Nu-Serum rather than the foetal calf serum used in other laboratories. It was thought that the high transformation frequencies observed with Nu-Serum were due either to endotoxins or to growth factors in the serum. Experiments showed that endotoxins were not the cause.

Subsequently problems were encountered with poor growth, low plating efficiency and an increase in granularity in the cells. An infection was suspected and extensive experiments were carried out to trace it, but with negative results. It now appears that the Nu-Serum must have deteriorated. In future, all experiments will be carried out using foetal calf serum.

IV. Objectives for the next reporting period:

To ascertain the role of specific growth factors in the transformation of 10T½ cells, with a view to improving the reproducibility and sensitivity of the assay.

To establish whether an inverse dose-rate effect exists for a fission-like neutron spectrum.

To establish whether there is a difference between the effectiveness of α particles and protons at an LET of about 20 keV μm^{-1}.

To set up and characterise an assay for transformation of rat NRK (epithelial) cells based on growth in soft agar.

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

The MRC Radiobiology Unit, Chilton (Dr D T Goodhead) 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 National Radiological Protection Board, Chilton, and the Central Electricity Generating Board, Berkeley Nuclear Laboratories.

VI. Publications:

1. C.J. Roberts, D.T. Goodhead, G.R. Morgan and P.D. Holt (1985) Transformation of C3H 10T½ with ^{238}PU alpha particles. (Abstract) Proc. 8th International Congress of Research Vol. 1, p.182.

2. C.J. Roberts and D.T. Goodhead (1987) The effect of ^{238}Pu alpha particles on the mouse fibroblast cell line C3H 10T½: characterization of source and RBE for cell survival. Int. J. Radiat. Biol. 52, 871-882.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Institut Curie
Section de Biologie
Rue d'Ulm, 26
F - 75321 Paris Cédex 05

Contract no.: B16-E-151-F

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

Complementation analysis by cell fusion in Fanconi's anemia. Search for complementation of defective repair functions by DNA transfection.

Head(s) of project: **Dr. Ethel MOUSTACCHI**

Scientific staff:

C. Diatloff-Zito
D. Papadopoulo
S. Nocentini
S. Rousset
D. Averbeck
D. Fraser
B. Porfirio
F. Rosselli
E. Moustacchi

I. Objectives of the project: Fanconi's anemia (FA) belongs to the class of human hereditary diseases characterized by chromosomal instability, cancer proneness and hypersensitivity to physical and/or chemical agents. This hypersensitivity to environmental chemical mutagens and/or to radiations is thought to be related to a defect in the processing of DNA lesions. FA cells are hypersensitive to DNA cross-linking agents. Mouse lymphoma cells mutants (MC^S) sharing with FA several phenotypic features, have been isolated. Since the molecular steps of DNA interstrand crosslinks removal is not yet fully unterstood in mammalian cells, FA and MC^S mouse cells can serve as "mutant" systems in comparison to normal cells to unravel this mechanism. More important, the cloning of the gene(s) involved in the processing of DNA interstrand crosslinks is necessary for the definition of the number of genes, their expression, regulation and chromosomal localisation. In order to reach this goal, correction of the FA fibroblasts and mouse MC^S lymphoblasts defect by transfection with high molecular weight DNA constituted a first step.

II. Objectives for the reporting period:

1. Transfection of human FA fibroblasts with rodent DNA and preparation of a genomic library in λ phage. Complementation of the different functions defective in FA (cytotoxicity, chromosomal instability, rate of DNA semi-conservative synthesis). Adaptation of the electroporation method of transfection to mouse lymphoblastoïd MC^S mutants using a cDNA library in plasmid or cosmid DNA. Analysis of secondary transfectants.

2. Further characterization of FA cell lines belonging to the genetic complementation groups A and B and of MC^S mouse mutants : sensitivity to specific types of monoadducts, influence of the presence of such monoadducts on the repair of crosslinks, fate of crosslinks using different methods.

III. Progress achieved:

1) Toward the cloning of gene(s) involved in the response to DNA crosslinking agents, definition of the conditions for transfection of DNA in mammalian cells.

a) Correction of the hypersensitivity to mitomycin C (MMC) of FA fibroblasts by transfection with mouse DNA. In 1986, we have shown that the MMC-sensitivity of FA primary skin fibroblasts can be fully corrected by transfection of high molecular weight DNA from normal human cells (Proc. Natl. Acad. Sci. USA, **83**, 7034). This has been recently confirmed with respect to sensitivity of FA fibroblasts to diepoxybutane (Shaham et al., Proc. Natl. Acad. Sci. USA, 1987, **84**, 5853). The correcting DNA human sequences cannot be distinguished from the host FA genome. It is why we have attempted this year to correct the FA defect with DNA from mouse cells for which specific probes are available. The previously devised protocol was adopted. We show that : i) FA fibroblasts from complementation group B (FA 145) can be complemented to an almost normal response to MMC by transfection of DNA from L5178Y mouse lymphoma cells. Several FA 145 stable transfectants containing different amounts of mouse DNA repeated sequences, as determined by Southern blotting, have been generated. A genomic library in λ phage has been prepared and is actually screened. ii) FA fibroblasts from complementation group A which, as we have shown, are the most defective both in terms of clonogenic cell survival to MMC and of crosslinks repair (Mut. Res., 1987, **184**, 271), cannot be corrected by transfection with DNA from mouse L5178Y cells in spite of several trials in a variety of conditions. However correction (partial but stable) takes place when total embryonic C_3H mouse DNA is used. These date raise the possibility that DNA from cultured lymphoma and from embryonic mouse cells are not in the same state of competence for expression of a gene necessary for the processing of crosslinks in FA group A cells.

These observations show that DNA sequences correcting the FA defect are present not only in human normal DNA but also in mouse DNA.

b) DNA transfection into MC^S_I mouse mutant cells by electroporation. MC^S mouse mutants share with FA hypersensitivity to DNA crosslinking agents. Electric pulse mediated gene transfer, electroporation, has been chosen for introducing DNA sequences into such cells which grow in suspension and cannot in our hands be transfected by the Ca-PO_4 precipitation technique. As a first step, the conditions for the electroporation of the pSV_2 neo plasmid in the MC^S_I mouse mutant (from complementation group I) have been defined. The influence of parameters including electric field characteristics and pulse length on MC^S_I cells viability and permeabilisation (quantified by lucifer yellow fluorescence of cells) were studied. Different concentrations of pSV_2 neo plasmid DNA, as well as different expression times of the neo gene (expressed as acquired resistance to the G 418 antibiotic) and selection conditions, were tested. As a result, a transfection efficiency of 4.10^{-6} of the neo gene was obtained with an optimal concentration of 50 ug/ml of plasmid DNA using 3 pulses of a square wave electric pulse field at 1.5 kV/cm and a frequency of 1 Hz (electropulsator ATEM-CNRS). Stable integrants clones were isolated and molecular analysis of DNA from G 418 resistant cells reveals that all clones tested contain one copy of the integrated plasmid. Electroporation with a cDNA human library in a pcD2 vector (Okayma) is now under study.

2) Further characterization of FA cells from complementation groups A and B.

It has been claimed that the primary defect in FA may be in the incision of interstrand crosslinks (CL) but this is not supported by the work of others. This is why we have reconsidered this problem taking into account the fact that the genetic and phenotypic heterogeneity, recently demonstrated in FA, could underlie the response to DNA crosslinking agents. In view of the controversy about the nature of the defect in FA, we used a variety of methodologies. Psoralens were chosen as crosslinking agents since they offer several advantages : a) treatments can be accurately controlled by the activating UVA light, b) the type of lesions are well

defined as monoadducts (MA) on pyrimidines and interstrand CL, c) by the use of different wavelengths the photoreaction can be directed towards the induction of either mixtures of MA and CL at various ratios (365 nm) or of solely MA (405 nm).

a) Fate of crosslinks. As reported last year alkaline elution analysis of the fate of 8-methoxypsoralen (8-MOP) photoinduced CL in FA fibroblasts from complementation groups A and B in comparison to normal cell lines showed that incision of CL takes place in FA cells but it is somewhat hampered in terms of kinetics and of final amounts of CL incised. FA group A appeared to be more defective that group B cells. These observations have been extended to more fibroblastic cell lines and to lymphoblastoid cells. These conclusions are supported by another approach relying on the visualization by electron microscopy of CL. Indeed, the results show a dose-effect relationship for the induction of CL and after 24 h post-treatment incubation, the proportion of crosslinked fragments is significantly reduced in all cell lines examined. The removal is of about 50% in normal cells and is lower in FA cells, again more markedly in group A cells. The evaluation of the CL frequencies with regard to DNA fragment length confirms that FA cells accomplish as normal ones the first incision step of CL but they are somewhat affected in the efficiency of this process. Studies using 4,5',8-trimethylpsoralen (TMP), a psoralen derivative with a higher photoaffinity for DNA than 8-MOP, and of a double irradiation protocol (405 nm plus 365 nm), confirm that incision of CL is systematically hampered in FA group A cells compared to normal. This is true for a constant number of total adducts measured by the photobinding of ^{3}H-TMP to cellular DNA and different ratios of CL over MA. Also when the proportion of CL over MA is increased, the differential sensitivity in terms of cytotoxicity between FA group A and normal cells increases.

b) Fate of monoadducts. In conditions in which solely MA are induced, i.e. treatment with TMP and 405 nm radiation, FA cells appear to be more sensitive in terms of cytotoxicity than normal cells. FA group B cells are 5 fold, whereas group A cells are 3 fold more sensitive than normal cells. However, after treatments inducing a mixture of MA and CL (TMP or 8-MOP plus 365 nm), FA group B cells are less sensitive than group A cells. Both FA lines were found to be less able than normal ones to repair TMP plus 405 nm induced crosslinkable MA (i.e. furan side). During a 24 h post-treatment incubation, FA cells from the two complementation groups have incised 4 times less MA than normal cells.

Normal and FA group A cells are more sensitive to TMP plus 365 nm than to TMP plus 405 nm treatments. FA 150 cells are more sensitive than normal cells to the crosslinking component in the mixture of lesions induced at 365 nm. Surprisingly, FA 145 (group B) cells appear to be essentially sensitive to the TMP-photoinduced MA at 405 nm. This is in line with the fact that following treatment with 8-MOP and 365 nm, FA group B cells recover a normal rate of DNA semi-conservative synthesis. With the exceptions of methylnitrosoguanidine and this new finding on TMP photoinduced MA, FA cells are not sensitive to monofunctional agents or radiations. It appears that distorsions introduced in the DNA structure by specific MA can be recognized by the incision complex as CL.

3) Further characterization of the MC^{S} mouse mutants.

In collaboration with Drs. Hama-Inaba and Sato, we show that mitomycin C-sensitive mutants MC$^{S}_{I}$ and MC$^{S}_{II}$ are also highly sensitive to the lethal effect of other DNA crosslinking agents such as photoaddition of 8-MOP or cis-diamminedichloroplatinum II (cis-DDP). They respond almost like normal mouse cells to photoaddition of a monofunctional compound like 3-carbethoxypsoralen (3-CPs) or to trans-DDP. Incorporation levels of labelled 8-MOP or 3-CPs in wild type and the two mutants were the same, indicating that the sensitivity is not caused by differential incorporation of the agents. Crosslink repair was studied by alkaline sucrose gradient sedimentation. Normal and mutants cells from both complementation groups are able to incise crosslinked DNA. A situation similar to that found in FA is observed.

IV. Objectives for the next reporting period:

Using the different strategies defined in 1987, we should be in a good position for the cloning and molecular characterization of the gene(s) involved in the processing of crosslinks in both FA and mouse mutants hypersensitive to crosslinking agents.

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

- Dr. E. Gluckman (Unité fonctionnelle de greffe de moelle, Département d'Hématologie, Hôpital Saint-Louis, Paris, France).
- Prof. J. A. Heddle (Department of Biology, York University, Toronto, Canada).
- Drs. H. Hama-Inaba and K. Sato (Division of Genetics, National Institute of Radiological Sciences, Chiba, Japan).

VI. Publications:

- Two complementation groups of Fanconi's anemia differ in their phenotypic response to a DNA-crosslinking treatment.
  E. Moustacchi, D. Papadopoulo, C. Diatloff-Zito and M. Buchwald.
  Hum. Genet. (1987) 75 : 45-47.

- The fate of 8-methoxypsoralen-photoinduced DNA interstrand crosslinks in Fanconi's anemia cells of defined genetic complementation groups.
  D. Papadopoulo, D. Averbeck and E. Moustacchi.
  Mutation Res. (1987) 184 : 271-280.

- L'anémie de Fanconi : aspects génétiques et moléculaires.
  E. Moustacchi et C. Diatloff-Zito.
  Médecine-Sciences (1987) 3 : 608-612.

- Sequence context effects of 8-methoxypsoralen photobinding to defined DNA fragments.
  E. Sage and E. Moustacchi.
  Biochemistry (1987) 26 : 3307-3314.

- DNA repair in yeast : genetic control and biological consequences.
  E. Moustacchi.
  In "Advances in Radiation Biology" (1987) Ed. J. T. Lett, Academic Press, San Diego, Calif., vol. 13, pp. 1-25.

(within the frame of our 1981-1985 CEE program for this last reference. Due to long

delay in publication of this book).

Thesis

- Cartographie des photoadduits de psoralènes sur des séquences définies d'ADN.
  V. Boyer.
  Diplôme d'Etudes Approfondies de Génétique et Physiologie, Université de Paris XI, Juin 1986.

- Transfection par électroporation du gène neo dans des cellules lymphoblastoïdes de souris mutantes sensibles aux agents de pontage de l'ADN.
  A. Lassailly.
  Diplôme d'Etudes Approfondies de Bases Fondamentales de l'Oncogénèse, Université de Paris VII, Octobre 1987.

Title of the project no.: 2

Head(s) of project: Dr. J. COPPEY

Scientific staff: J. Coppey
B. Lopez
M. Sala-Trepat

I. Objectives of the project: The hypersensitivity of Fanconi's anemia (FA) cells to DNA cross-linking agents can be due to a defect in the processing of cross-links. We hope to get instructive data on the recombinational repair pathway for cross-linked DNA in FA compared to normal fibroblast cells by analysing the process of multiplicity reactivation (MR) of trimethylpsoralen damaged Herpes virus (TMP-HSV) and the fidelity of the reactivation. Moreover the use of an in vitro system allowing to monitor events of homologous recombination promoted by human nuclear extracts may allow to dissect the possible abnormalities of this process in FA cells.

II. Objectives for the reporting period:

1. Completion of the multiplicity reactivation analysis in several cell lines from five FA and three normal donors.

2. Analysis of the phenotype of a representative group of thymidine kinase minus (tk^-) mutants, 10 mutants per each cell line (2 normal and 3 FA).

3. Setting of an in vitro system allowing to monitor molecular steps of homologous recombination and of recombinational repair of double stranded breaks.

III. Progress achieved:

1. As already mentionned in the preceeding report, we observe that all FA lines tested exhibit a slight defect in host cell reactivation of TMP-HSV. In addition over a limited range of TMP photoaddition to virus (leaving survival > 10^{-3}) MR of TMP-HSV is significantly more pronounced in FA than in normal host cells.

Concerning the rate of forward mutation towards a tk^- phenotype :

- the rate is lower in the FA (3 lines) than in the normal cell lines (3 lines) in the progeny from untreated virus
- in the progeny from (TMP-HSV) reactivated by multiplicity of infection, the rate is either increased (3 normal lines) or unchanged (5 FA lines) compared to that from control virus.
- the MR effect for TMP-HSV can be ascribed to either cross-links or to monoadducts formed by covalent binding of psoralen to viral DNA. Attempts aimed at increasing the relative proportion of cross-links by two successive exposures to UV-A reveals that following contact in the dark of HSV with TMP, a fraction of molecules ($\simeq$ 20%) remains trapped in viruses. It is therefore not feasible to create only TMP cross-links in viral DNA.

2. A phenotypical analysis of 50 individual tk^- mutant viruses following their expansion in mini stocks onto CV-1 monkey cells is performed for three gene products corresponding to loci adjacent to each other in the genetic map of HSV : tk gene (iododeoxycytidine ICdR resistance), DNA polymerase gene (phosphonoacetic PAA sensitivity) and glycoprotein B (sensitivity to thermal inactivation). The underlying assumption was that the existence of important deletions/local rearrangements in mutant viral DNA could result in phenotypical alterations in the expression of adjacent genes in some mutant viruses.

All mutants from virus replicated in either normal or FA host cells present comparable complete ICdR resistance, and sensitivity to PAA as well as to thermal inactivation both similar to that of wild virus (Coppey et al., submitted).

3. The phenomenon of increased MR in FA cells probably reflects alteration(s) in the processing of TMP adducts in viral DNA : cross-links and/or monoadducts responsible for lethal effect, for instance those giving rise to overlapping tracks of excision. Full repair of either damage involves events of recombination with a coinfecting viral partner undamaged in the homologous sequence.

This possibility led us to devise an in vitro system allowing to study the steps of homologous recombination catalysed by semi-purified human nuclear extracts. We indeed observe that extracts from human transformed cells (3 epithelial lines ; one lymphoblastoïd line) contain an activity promoting targeted replacement of an altered gene sequence in the replicative form (RF) of phage M13 mp8 by the functional one arising from a restriction fragment isolated from wild M13 mp8. Molecular analysis of the recombination intermediates show that the process takes place by single strand exchange (Lopez et al., 1987). The gene replacement is error-free, probably because the recombining DNA's are transiently protected against nuclease attack (Lopez and Coppey, submitted). The repair of a double strand break in the RF occurs preferentially via a recombinational pathway with an homologous intact DNA sequence (Lopez and Coppey, 1987).

IV. Objectives for the next reporting period:

We now aim at using this system to study the characteristics of these recombinase activities in lymphoblastoïd cell lines from FA and normal donors.

However, an alternate possibility which could account for the increased MR in FA cells could be a particular proneness of coinfecting viral DNA's to recombine to each other without the need of any altered recombinase activity in these cells. It must be pointed out that the first half-incision of cross-links is slowed down in FA cells (Papadopoulo et al., 1987) : if this holds true in cross-linked viral DNA, it would produce a greater number of single stranded DNA patches per time unit in FA than in normal cells. Such damage (transformed or not into double strand breaks/gaps) is indeed recombinogenic per se (Lopez and Coppey, 1987).

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

VI. Publications:

- Lopez B., Rousset S. and Coppey J. (1987) Homologous recombination intermediates between two duplex DNA catalysed by human cell extracts. Nucleic Acids Res., 15, 5643-5655.

- Lopez B. and Coppey J. (1987) Promotion of double strand break repair by human nuclear extracts preferentially involves recombination with intact homologous DNA. Nucleic Acids Res., 15, 6813-6826.

- Lopez B. and Coppey J. Molecular analysis of homologous recombination catalysed by human cells extracts : fidelity and DNase protection (submitted).

- Coppey J., Sala-Trepat M. and Lopez B. Multiplicity reactivation and mutagenesis of trimethylpsoralen-damaged Herpes virus in normal and Fanconi's anemia cells (submitted).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Freie Universität Berlin
Altensteinstrasse 40
D - 1000 Berlin 33

Contract no : BI6-E-223-D

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

Prof. Dr. G. Obe
Institut für Genetik
Freie Universität Berlin
Arnimallee 5-7
D - 1000 Berlin 33

Telephone number: 030-838.36.41

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.: B 16 - 0223 - D (B)
Evaluation of the frequencies of chromosomal aberrations induced in human blood lymphocytes by low doses of X-rays

Head(s) of project:
Prof. Dr. Günter Obe

Scientific staff:

I. Objectives of the project:
The programme is aimed to accurately estimating the frequencies of dicentric chromosomes in human peripheral blood lymphocytes treated in vitro with very low doses of X-rays (0 and 3 rad).

II. Objectives for the reporting period:
Scoring of preparations made from irradiated human peripheral lymphocytes.

III. Progress achieved:

Irradiation, physical dosimetry and chromosomal preparations of lymphocytes was made at the National Radiological Protection Board, Chilton, U.K., and coded preparations were sent to the different participating laboratories.

Up to now about 10,000 metaphases were scored with respect to all aberrations occuring. Fotos were taken from all metaphases with dicentric chromosomes, ring chromosomes or chromatid translocations. The fotographs shall be used for karyotyping the aberrant cells to see if chromosomes are nonrandomly involved in the formation of aberrations.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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, G. BOSI, G. MARZIALI, I. SAGGIO

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 interaction between low doses of ionizing rdiations with different chemical mutagens e.g. Bleomycin, Mitomycin C, Methylmethane sulphonate, or with a subsequent chromic treatment with ionizing radiations using ^{3}HdThd.

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 to high doses of the agent to be tested: mitomycin (MMC), which induces cross-links in DNA, bleomycin, an S-independet radiomimetic agent that induces double-strand breaks in DNA; and methyl methanesulfonate (MMS), an S-dependet agent that alkylates DNA and then leads to single-strand breaks. Cells were also exposed to the radiation from high doses of ^{3}H dThd to see if the damage induced by the particles from the disintegrating tritium is also repairable. The cells were scored to see if the prior exposure reduced the number of chromatid and isochromatid breaks induced by the challenging doses.

Whole blood (0.5 ml) from donors of both sexes was added to 4.5 ml of RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2% phytohemagglutin M (Gibco). When ^{3}H dThd was used to adapt the cells 3.7×10^{3} Bq/ml (0.1 µCi/ml) ^{3}H dThd (sp. act. 2.5×10^{11} Bq/mmol, i.e., 6.7. Ci/mmol, New England Nuclear) was present either from 8 h of culture until the time of fixation, or from 24 to 36 h of culture according to the given experiment. When 0.01 Gy of X rays (250 kVp, 15 mA, 1 Gy/min, halfvalue layer 1.06 mm Cu, from a Philips RT250 therapeutic unit) was used as the adapting dose, it was administered at 24 h of culture. The cells were subsequently challenged with freshly made aqueous solutions of various agents. After the challenging agents were added for various times, 0.1 ml of Colcemid (final concentration 2×10^{-7} M) was added to each culture, which was then fixed 2 h later in methanol-acetic acid (3:1), after firts being exposed to a hypotonic solution of 0.075 M KCl to spread the chromosomes. Two parallel cultures were used for each point. One hundred cells were scored for chromatid aberrations at each point, except in the experiment with MMC (Kyowa), in which 200 or 300 cells were scored per point.

RESULT AND DISCUSSION

When cells exposed to 0.01 Gy of X rays or to low doses of tritiated thymidine were subsequently challenged with high doses of tritiated thymidine or bleomycin,which can induce double-strand breaks in DNA, or mitomycin C, which can induce cross-links in DNA, approximately half as many chromatid breaks were induced as expected. When, on the other hand, the cells were challenged with the alkylating agent methyl methanesulfonate (MMS), which can produce single-strand breaks in DNA, approximately twice as much damage was found than was induced by MMS alone. The results indicate that prior exposure to 0.01 Gy of X rays reduces the number of chromosome breaks induced by doublestrand breaks, and perhaps even by cross-links, in DNA, but has the opposite effect on breaks induced by the alkylating agent MMS. The response was markedly greater if the initial exposure was to ^{3}H dThd rather than to 0.01 Gy of X rays. Furthermore, the synergism evidently occurs over a range of challenging doses of MMS, because when the dose was varied by changing the concentration of the chemical and/or by changing the exposure time, synergism still occurred. The possibility that the change in response from a decrease in yield to synergism is the results of changes in some undefined culture conditions can be ruled out by the positive controls carried out at the same time with ^{3}H dThd as the challenging agent. In these positive controls, the decrease in aberration yield was still observed.

The present experiments, which show that the adaptive response elicited by exposure to ionizing radiations leads to increased damage when cells are exposed to MMS, offer, further proof that the mechanism of the adaptation is different from that induced by initial exposures to low doses of alkylating agents, which in bacteria, at least, induce an alkyl transferase. The synergism found here after challenges with the alkylating agent MMS, and the great differences in the types of DNA lesions induced by ionizing radiations and alkylating agents, indicate that different mechanism are involved in the adaptation induced by the differing agents.

IV. Objectives for the next reporting period:

To study the individual variability of human lymphocytes in induction of "adaptive response"

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:
"Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiations as well as to chemical mutagens that induce double-strand breaks in DNA" Int. Jour. Rad. Biol. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.. 1. 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: In order to estimate the frequency of chromosomal aberrations in human peripheral blood lymphocytes treated in vitro with low doses of x-rays,blood from 20 donors has been used. This research will give informations about variations in sensitivity of donors at low doses. Futhermore all cells containing exchange type aberrations will be photographed and karyotyped in order to determine wether certain chromosomes are more frequently involved in such aberrations.

II. Objectives for the reporting period: To score the encoded slides from the human lymphocytes of 20 donors irradiated in vitro at Chilton with 0,5,30 and 300 mGy of 169 KeV ISO wide series x-rays.

III Progress achieved:

Methods. Lymphocytes cultures from 10 male and 10 female healthy donors aged 20-40y have been irradiated acutely with o,5,30 and 300 mGy. Slides prepeared at Chilton (GB) were coded and distributed to all partecipants.

Results. Until now approximately 70% of our share of the scoring has been completed by the end of 1987.

Discussion. Until the decoded data of the scoring from all the laboratories will not be examined and statistically evaluated we shall not know the results.

IV. Objectives for the next reporting period: To complete the scoring of the coded slides and to examine the results with the other partecipants in order to decide wether it is necessary to score more doses. The cell containing exchange-type aberrations will be karyotyped for evidence of non-random involvement of chromosomes in exchanges.

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

1. State University of Leiden,Netherland (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. National Radiological Protection Board,UK (Dr.Lloyd)

VI. Publications:

D.C.Lloyd,A.A.Edwards,A.Lèonard,Gh.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.Radiat.Biol. (in press)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no: BI6-E-154-F

Université Paris VII
Institut Jacques Monod
Place Jussieu, 2
F - 75251 Paris Cédex 05

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 n° :

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 M. PETRANOVIC, Dr R. RAYSSIGUIER, Dr R. WAGNER

I. Objectives of the project :

(a) Physical mapping and quantification of mutations in any DNA : isolation of mismatch-recognizing proteins and the study of the structure of diverse DNA base pair mismatches in defined oligonucleotides.

(b) Molecular mechanisms and genetic control of radiation-induced mutagenesis through (i) misreplication of the DNA lesion and (ii) radiation-stimulated gene conversion.

II. Objectives for the reporting period :

(i) Mismatch repair of deaminated 5-methyl-cytosine (5-meC) in Escherichia coli, xenopus laevis eggs and human HeLa cells.

(ii) Molecular basis for genetic alterations involving hyperrecombination or hyperconversion.

(iii) In vitro mismatch repair in extracts of frog egg and human HeLa cells.

(iv) Oncogenic DNA replication errors in the human K-ras codon 12 sequence: in vitro studies with mammalian cell extracts (collaboration with Dr B. Dimitrijevic's group in Belgrade).

(v) Mutagenic replication of a defined DNA lesion (collaboration with Dr M. Goodman's laboratory, USC, Los Angeles).

III. Progress achieved :

1. Methodology.

Genetic manipulations of E. coli and its viruses λ, M13 and φx174 ; DNA strand separation, heteroduplex formation in vitro, in vitro packaging of heteroduplex DNA, oligonucleotide synthesis and directed mutagenesis, oligonucleotide heteroduplexes mimicking oncogenic replication errors, NMR analyses of DNA mismatch structure, detection of mismatch repair in vitro by localised specific DNA degradation and repair synthesis, physical mapping of repair events, detection of single mismatches in DNA. Preparation of nuclear extracts from yeast, HeLa cells and Xenopus egg and enzyme analyses.

2. Results.

Mismatch repair systems in E. coli act to repair mismatched bases arising as errors in DNA replication, 5-meC → T deamination and in genetic recombination involving homologous but non identical sequences (review, ref. 1).

(i) Mismatch repair of deaminated 5-methyl-cytosine (5-meC) in Escherichia coli, Xenopus laevis eggs and human HeLa cells.

We have described in detail a specialised mismatch repair system which functions to correct 5-meC → T deaminations (spontaneous or induced by heat or ionizing radiation) in E. coli (ref. 2,3). Fig. 1 illustrates its action. The system removes very few (perhaps only one) bases (ref. 3), hence its name very-short-patch (VSP) repair, and is highly specialised to act only on G:T mismatches that can arise by 5-meC→T deamination by repairing them to G:C and thus restoring the original sequence.

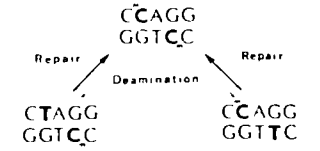

Figure 1. [illegible]

Mammalian DNA contains 10 to 100 fold higher percentage of 5-meC than E. coli DNA, and the genome is 1000-fold larger, thus an evident need for a VSP-repair analog in vertebrates. We have detected efficient mismatch repair in vitro in Xenopus egg and HeLa cell extracts using specially designed heteroduplex substrates that mimic 5-meC→T deamination in eukaryotic DNA (e.g. 5' C T G G → in M13 phage DNA) using restriction enzymes to diagnose repair events G:T → G:C (MspI) and G:T → A:T (BstNI) (ref. 4).

(ii) Molecular basis for genetic alterations involving hyperrecombination or hyperconversion.

Genetic (DNA sequence) alterations can arise either de novo, by newly arising errors in DNA replication, or de antiquo by scrambling, by recombination, pre-existing DNA sequences. We have demonstrated the molecular mechanism by which the VSP repair generates sequence diversification whereas the long patch (LP) repair generates conservation, or homogeneization, of the parental DNA sequences (réf. 3). There are multiple impacts of these findings to the genetics of all diversified repeated genes (families) e.g. immunoglobulin, histocompatibility, diverse receptors and oncogene systems. Ionizing radiation known to be a poor base substitution induced in single copy genes, may be a potent base substitution inducer in multigene families.

(iii) In vitro mismatch repair in extracts of frog egg and human HeLa cells.

We have provided first evidence for mismatch repair in vitro by extracts of nuclei of cells from vertebrates. The repair involves recognition of the mismatch and its repair involving a localised DNA synthesis (réf. 5). This repair can be involved either in correction of replication errors (conservation) or in gene conversion (diversification).

(iv) Oncogenic DNA replication errors in the human K-ras codon 12 sequence : in vitro studies with mammalian cell extracts (collaboration with Dr B. Dimitrijevic's group in Belgrade).

About 30% of human tumours carry a mutation in the K-ras oncogene. The two G:C pairs of the codon 12 (G G T) are prime targets for substitution mutations. We have constructed synthetic oligonucleotides mimicking replication errors G:T; A:C; C:C and G:A : both Pu:Py mismatches are well recognized and degraded by nuclear extracts of rat liver cells whereas the G:A and C:C mismatches were unrecognized. This specificity is consistent with the nature of these mutations found in spontaneous tumour and suggests that the majority of codon 12 K-ras mutations in general population may arise by replication error rather than by DNA damage (réf. 6).

(v) Mutagenic replication of a defined DNA lesion (collaboration with Dr M. Goodman's laboratory, USC, Los Angeles). Base-loss sites may be the most frequent and ubiquitous spontaneous and induced DNA lesion. A defined

oligonucleotide with one base-loss site at a specific plaxe is used to monitor quantitatively and qualitatively the SOS-induced process which permitts E. coli DNA polymerase III to copy such site. Preliminary results indicate inducible factors altering DNA polymerase III thus enabling it to copy the lesion and fix the mutagenic event. The same test can be used to test nuclear extracts from irradiated eukaryotic cells.

IV. Objectives for the next reporting period :

(a) Adaptation of the experimental conditions from our recent work (Brooks et al.) for the routine detection of mismatched base pairs by the Xenopus egg extract.

(b) Development of chemical and immunological probes for detection of unrepairable mismatches escaping detection in (a).

(c) Biochemical characterization of Xenopus and human mismatch repair systems.

(d) Biochemistry of mutagenic "bypass" replication.

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).

Drs 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. and R. Wagner : "Mismatch repair". Annual Rev. of Genetics (1986) 20, 523-538.

2. Jones, M., Wagner, R. and M. Radman : "Mismatch repair of deaminated 5-methyl-cytosine". J. Mol. Biol. (1987) 194, 155-159.

3. Jones, M., Wagner, R. and M. Radman : "Mismatch repair and recombination in E. coli". Cell (1987) 50, 621-626.

4. Brooks, P., Dohet, C., Petranovic, M. and M. Radman : "Mismatch repair in Escherichia coli and in Xenopus egg extracts". In : Eds Friedberg, E.C. and Hanawalt, P. "Mechanisms and consequences of DNA damage processing", Alan Riss Publ. (1988) in press.

5. Brooks, P., Dohet, C., Almouzni, G., Méchali, M. and M. Radman (1988)

"Mismatch repair involving localised DNA syntheis in Xenopus egg extracts" submitted to Cell.

6. Dimitrijevic, B., Bozin, D., Sunjevaric, I., G. Cerovic and M. Radman (1988) "Recognition of mismatched base pairs at the site of oncogenic K-ras gly-12 mutations by a mammalian cell extract", submitte d to Nature.

7. Dohet, C., Dzidic, S., Wagner, R. and M. Radman : "Large non-homology in heteroduplex DNA is processed differently than single base pair mismatches". Mol. Gen. Genet. (1987) 206, 181-184.

8. Jones, M., Wagner, R. and M. Radman : "Repair of a mismatch is influenced by the base composition of the surrounding nucleotide sequence". Genetics (1987) 115, 605-610.

9. Längle-Rouault, F., Maenhaut-Michel, G. and M. Radman : "GATC sequences, DNA nicks and the muth function in Escherichia coli mismatch repair". EMBO J. (1987) 6, 1121-1127.

10. Radman, M. : "Stabilisation et diversification de l'information génétique: impact sur la cancérogénèse". In : LE CANCER (Les Presses de l'Académie internationale de Lutèce) (1987) 91-101.

11. Radman, M. : "DNA methylation and mismatch repair : molecular specificities". In: W. Guschlbauer and W. Saenger, eds. "DNA-ligand interactions from drugs to proteins" pp. 217-224. Plenum Press, New York & London (1987).

12. Radman, M. : "Maintenance and diversification of genetic information". In : "Genetics of industrial microorganisms". M. Alacevic, D. Hranueli and Z. Toman, eds. pp 3-10. Pliva-Zagreb (1987).

13. Quinto, I. and M. Radman : "Carcinogenic potency in rodents versus genotoxic potency in E. coli: a correlation analysis for bifunctional alkylating agents". Mut. Res. (1987) 181, 235-242.

14. Radman, M. : "DNA repair and genetic alterations", In "New Trends in Genetic Risk Assessment". G. Jolles and A. Cordier Eds. (Academic Press Publ.) (1988), in press.

15. Radman, M. : "Mismatch repair and genetic recombination". In: "Genetic recombination", eds. R. Kucherlapati and G.R. Smith. "Mismatch repair

and genetic recombination". American Soc. of Microbiol. (1988), in press.

15. Quinto, I., Tenenbaum, L. and M. Radman : "Genotoxicity profile of monofunctional alkylating agents in E. coli : quantitative correlations with carcinogenic potency in rotends". Proc. Natl. Acad. Sci. USA, sous presse.

17. Dzidic, S. and M. Radman : "Genetic requirements for hyperrecombination by very short patch mismatch repair : involvement of E. coli DNA polymerase I". Submitted to J. Bact.

18. Radman, M. and R. Wagner : "Correction of errors in DNA". Scientific American, in press.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
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B - 1640 Rhode St Genèse

Prof. J. Rommelaere
Lab.Biophysique et Radiobiol.
Université Libre de Bruxelles
rue des Chevaux 67
B-1640 Rhode St. Genèse

Telephone number: 02-358.35.30 (Lxt. 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 and mammalian cells. 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 mechanism of mutagenesis.

II. Objectives for the reporting period:

The objectives for 1987 were focused on : i) the genetic characterization of the mechanism of SOS mutagenesis using different mutants of E.coli, ii) the relationship between DNA replication fidelity, mismatch repair and the presence of lesions in the DNA and iii) the replication and mutagenesis of damaged DNA using in vitro damaged heteroduplex DNA containing as genetic markers one pair of mismatched bases.

III. Progress achieved:

1. Methodology

Different systems were developed for detecting mutations induced by ultraviolet irradiation in E.coli.

1°) Mutagenesis of the E.coli chromosome was determined by measuring either the apparition of mutants resistant to rifampicin or the reversion of a defined lac$^-$ mutation to the Lac$^+$ phenotype. The first system allows to detect essentially base substitution mutations in the gene coding for the ß-subunit of the RNA polymerase. The second system allows to detect frameshift mutations since the lac$^-$ mutant used is a (+ 1) frameshift mutant in the gene lacZ.
2°) Mutagenesis of phages with single or double stranded DNA was measured in two different mutation systems : i) a forward mutation system detecting in the cI gene of phage lambda all types of mutations (i.e. : base substitutions, frameshifts and others) and ii) a reversion mutation assay detecting base substitution by looking for revertants of amber mutations.

2. Results and Discussion

1)In E.coli, agents which damage DNA and interfere with DNA replication, such as ultraviolet or ionizing radiations, induce a diverse set of physiological responses ("SOS response"). Among these are the induction of functions that allow the bacteria to be mutated. These various responses have been shown to result from the induction of damage-inducible genes. Evidence indicates that such damage-inducible genes exist in mammalian cells (Schorpp et al. Cell 37 : 861, 1984), suggesting that the SOS response in E.coli may reflect a universal mechanism of mutagenesis. Our genetic approach aims to identify the molecular basis of SOS functions implied in mutagenesis. DNA damage that blocks the replication fork, promotes in E.coli a mutator activity which generates mutations also in undamaged DNA. This was shown by the enhancement of mutagenesis of undamaged phage. The SOS mutator effect can also be promoted by mutation in the recA gene. Indeed modification of the RecA protein by the recA730 mutation increases the level of spontaneous mutations in the bacterial DNA. We have shown that this number of recA730-induced mutations is greatly increased in mismatch repair deficient strains in which replication errors are not corrected. This suggests that a majority of recA730-induced mutations (90%) arise through correctable, i.e. non-targeted replication errors. The recA730 mutation increases poorly spontaneous mutagenesis of phage lambda. UV-irradiation of recA730 host bacteria increases phage untargeted mutagenesis to the level observed in UV-irradiated recA$^+$ strains. The UV-induced mutator effect in recA730 mutants is not suppressed by an umuC mutations whereas the recA730-induced mutator effect is totaly abolished by mutation in this gene. Therefore UV and the recA730 mutations seem to activate different SOS mutator activities both generating untargeted mutations. Moreover, these mutator activities would have different specificities. The UV-induced untargeted mutagenesis mechanism induces both frameshift and base substitution mutations whereas the recA730-induced mechanism mainly induces base substitution untargeted mutations. Our results provide arguments favoring the hypothesis of a SOS-induced error-prone replication triggered by the activation of the RecA protein that increases the level of untargeted mutations (P. Caillet-Fauquet and G. Maenhaut-Michel, 1988, Mol. Gen. Genet., in press).

Polymerase I which is involved in DNA repair mechanisms and required in DNA replication for the scealing of Okazaki fragments in the lagging strands, was generally considered to be not essential for SOS mutagenesis. Indeed polA mutants are UV mutable. However, an altered form of DNA polymerase I, PolI* was purified from extracts of SOS-induced cells (Lackey et al., Proc. Natl. Acad. Sci. US 79, 330-334, 1982). There is a great similarity in the genetic requirements for the expression of PolI* and of phage lambda untargeted mutagenesis in SOS-induced cells. Therefore we studied untargeted mutagenesis of phage in well characterized polA mutants to determine if polymerase I plays a role in its expression. The results show that i) the polymerase but not the exonuclease 5'⟶3' activity of PolI is required for the expression of untargeted mutagenesis of the double stranded DNA phage lambda and of the single-stranded DNA phages ØX174 and M13 ; ii) proficient excision repair is required for double-stranded but not for single-stranded DNA phage ; iii) the umuC function which is not required for phage lambda untargeted mutagenesis is required for untargeted mutagenesis of the single-stranded DNA phages M13 and ØX174 (G. Maenhaut-Michel and P. Caillet-Fauquet, in preparation).

The differences observed in the genetic control of untargeted mutagenesis between single- and double-stranded DNA phages could reflect the importance of the nature of the DNA substrate for the function of the SOS mutator effect. This also suggests that the mutator effect functioning on cellular DNA is related to the one functioning on single-stranded DNA phages rather than to the one acting on the double-stranded DNA phage lambda.

ii) Involvement of the E.coli RecA protein functions in mismatch repair was tested by transfection of recA$^+$ and recA1 bacteria with heteroduplexes of phage ØX174 DNA. These heteroduplexes varied in the number of GATC sequences (zero, one or two), their state of methylation (fully methylated, hemimethylated or unmethylated) and in the nature and location of the mismatch (G:T or G:A). It was found that the recA1 mutation significantly decreases repair efficiency of both mismatches, but only in fully unmethylated heteroduplexes. The residual (nick-directed) mismatch repair in a mutH mutant was not affected by the recA1 mutation, suggesting that the MutH and RecA proteins may interact, directly or indirectly, in the recognition and/or the processing of the unmethylated GATC sequences (Länglé-Rouault and Maenhaut-Michel, submitted).

IV. Objectives for the next reporting period:

The molecular mechanism of the damage-stimulated DNA strand loss that could be responsible for the homozygocity of induced mutations in E.coli, will be studied. Heteroduplex DNAs, carrying sequenced mutations, will be irradiated or not with ultraviolet light or ionizing radiation and introduced by transfection, as single copies, into E.coli host cells. By analyzing the progeny of individual heteroduplex molecules we will determine the damage stimulated DNA strand loss using heteroduplexes containing a non-correctable mismatch base pair that allows genetic identification of each DNA strand.

Isolation and characterization of new mutants of E.coli affected specifically in the umuC/D independent pathway of SOS mutagenesis. Previous results have indicated that a second pathway of SOS mutagenesis exists in E.coli aside the pathway requiring the umuC/D gene products.

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

VI. Publications:

- Koffel-Schwartz, , Maenhaut-Michel, G. and Fuchs, R.P.P. (1987) Specific strand-loss in N-2-acetylaminofluorene modified DNA. J. Mol. Biol. 193, 651-659.
- Laenglé-Rouault, F., Maenhaut-Michel, G. and Radman, M. (1987) GATC sequences, DNA nicks and the MutH function in E.coli mismatch repair. EMBO J. 6, 1121-1127.
- Caillet-Fauquet, P. and Maenhaut-Michel, G. (1988) The nature of the SOS mutator effect : role of RecA protein in untargeted mutagenesis. Molec. Gen. Genet., in press.
- Maenhaut-Michel, G. (1988) Genetic characterization of the SOS mutator effect. Book of the ASM meeting on DNA Replication and Mutagenesis (november 8-12, 1987), in press.

Short communications:

- P. Caillet-Fauquet and Maenhaut-Michel, G. American society for microbiology, conference on DNA Replication and Mutagenesis. November 8-12,1987 on Marco Island, Florida. Nature of the SOS Mutator Activity:Genetic characterization of untargeted mutagenesis in E. coli.
- Maenhaut-Michel, G and P. Caillet-Fauquet. Genetic characterization of SOS induced-untargeted mutagenesis of phage:

Involvement of DNA polymerase I.
Mechanisms and consequences of DNA Damage Processing. January 24-31, 1988, UCLA, Taos, New Mexico.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-E-163-F

Centre National de la
Recherche Scientifique
Quai A. France, 15
F - 75700 Paris

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.: B 1 6 - 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.

Head(s) of project:
Dr. Alain SARASIN
Laboratory of Molecular Mutagenesis
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, Carlos F. MENCK, Michael R. JAMES, Catherine MADZAK, Anne STARY.

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) characterization of ultraviolet-induced mutation on SV40 virus.

b) Development of SV40-based shuttle virus.

c) Oncogene activation in Xeroderma pigmentosum tumors.

d) Structure of DNA ligase(s) in mammalian cells.

III. Progress achieved:

a) Characterization of ultraviolet-induced mutations on SV40 virus.

We have carried out an extensive study of UV-induced revertants of the temperature-sensitive tsB201 mutant. All the revertants are due to single or multiple base substitutions mapped on the late VP1 capsid genes. Some of the revertants have conserved the original tsB201 mutation. Almost all the mutation sites are located opposite putative pyrimidine-pyridimine lesions (pyrimidine dimers or pyrimidine (6-4) pyrimidones). In vitro treatment of UV-irradiated SV40 DNA with the E. coli photoreactivating enzyme in the presence of UV-A light strongly increases virus survival and decreases mutation frequency. This result analogous to that found in bacteria, indicates that both pyrimidine dimers and pyrimidine (6-4) pyrimidones are mutagenic in monkey cells.

At the same time, we have located and quantified the various UV-induced DNA lesions on a 342-bp SV40 DNA fragment in order to determine whether some rules may be define to predict the frequency of lesion formation as a function of the DNA sequence. The amount of pyrimidine dimers produced for a given site, is very dependent on the type of bases on the 5' and 3' adjacent sites while the amount of Py(6-4)Py is more dependent of DNA secondary structures. It is therefore very important to exactly quantify the frequency of lesions of a given DNA target since the overall frequencies will strongly depend of the DNA sequences.

b) Development of SV40-based shuttle virus.

The SV40-based shuttle virus, we have developed, consists of a plasmid which can replicate both in bacteria and in some mammalian cells. The shuttle genome can be packaged, without excision of plasmid sequences, into an SV40 pseudo-virion allowing us to use virus infection to transmitt the shuttle plasmid into COS monkey cells. Several targets to screen for mutations have been inserted : lacO, lac Z and supF genes.

All of them exhibit low spontaneous mutation frequency and can be used to characterize mutation spectrum of various DNA-damaging agents.

Pretreatment of COS cells with UV-light prior to infection with UV-irradiated shuttle virus increases significantly the mutation frequency. This result agrees with that found in the same cell system using animal virus as biological probe.

c) Oncogene activation in xeroderma pigmentosum tumors.

Xeroderma pigmentosum is a hereditary disease characterized by numerous epitheliomas on sun-exposed skin, due to defects of the excision-repair pathway. We have analyzed six skin tumors from XP children by the NIH 3T3 assay. Two of them gave rise to transformed foci due to the activation of the human N-ras oncogene. In these two tumors, the N-ras gene was overexpressed both in the original tumor tissues and in the NIH 3T3 transformed cells. Moreover, c-myc amplification and overexpression and Ha-ras gene amplification and rearrangements have been found in the same two tumors harboring an activated N-ras.

Experiments are in progress to screen more XP tumors and to try to define a pattern of oncogene activation in XP tumors in the aim to link the presence of unrepaired UV-induced lesions and the type of oncogene activation.

d) Structure of DNA ligase(s) in mammalian cells.

We have characterized the polypeptides showing DNA ligase activity in human and rat liver cells by using a combination of activity gels, DNA sequencing gels and label of the AMP-ligase complex. The so-called DNA ligase I has been found in every tissue with a M_r of about 130 kDa. This polypeptide can be partially proteolysed giving rise to smaller fractions between 110 to 75 kDa. The presence of the so-called ligase II has been difficult to ascertain and depends largely from the experimental protocols chosen. Our experiments do not completely support the idea that ligases I and II are coded by different genes.

IV. Objectives for the next reporting period:

a) Correlation between the type of UV-induced DNA lesions and UV-induced mutations in SV40 DNA.

b) Use of SV40-based shuttle virus to define mutation spectra.

c) Analysis of activated oncogenes in xeroderma pigmentosum tumors.

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.
Dr. H. SUAREZ, E.R. 278, Institut de Recherches Scientifiques sur le Cancer, B.P. n° 8, 94800 - VILLEJUIF.
Dr. Umberto BERTAZZONI, Istituto di Genetica Biochimica 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

VI. Publications:

. L. DAYA-GROSJEAN, M.R. JAMES, C. DROUGARD and A. SARASIN
Characterization of an immortalized xeroderma pigmentosum group c, cell line by an origin defective SV40.
Mutation Res., 183, 1987, 185-196.

. M. MEZZINA, J.M. ROSSIGNOL, PHILIPPE, M., IZZO, R., BERTAZZONI, U. and SARASIN A.
Mammalian DNA ligase : studies on the structure and fonction in rat liver tissues.
Eur. J. Biochem., 162, 1987, 325-332.

. C.F.M. MENCK, M.R. JAMES, A. GENTIL and A. SARASIN
Strategies to analyse mutagenesis in mammalian cells using simian virus 40 or shuttle vectors.
J. Cell Science, 1987, suppl. 6, 323-331.

. A. SARASIN, C.F. MENCK and M. R. JAMES
Shuttle vector/host systems for analysis of mutagenesis in mammalian cells.
Photobiochem. and Photobiophys., 1987, 343-351.

. C.F.M. MENCK, A. SARASIN and M.R. JAMES
SV40-based Escherichia coli shuttle vectors infectious for monkey cells. Gene, 1987, 53, 21-29.

. J. ARMIER, M. MEZZINA, M. LENG, R.P.P. FUCHS and A. SARASIN
N-acetoxy-N-2-acetylaminofluorene-induced damage on SV40 DNA : inhibition of DNA replication and visualization of DNA lesions.
1987, Carcinogenesis, In Press.

. H. SUAREZ, P. NARDEUX, Y. ANDEOL and A. SARASIN
Indepently alterated oncogene in human tumors.
Oncogene Res., 1987, 1, 201-207.

. A. SARASIN, F. BOURRE and A. GENTIL
Analysis of carcinogen-induced point mutations in a simian virus 40 genetic assay, Banbury Report, Cold Spring Harbor Symposium, 1987, In press.

. A. SARASIN and F. BOURRE
Use of SV40 to analyze DNA lesions and mutagenesis induced by ultraviolet light.
DNA repair, vol. 3., 1987 , In press.

. F. BOURRE, G. RENAULT and A. SARASIN
Sequence effect on alkali-sensitive sites in UV-irradiated SV40 DNA.
Nucl. Acid. Res. 1987, 15, 8861-8875.

. R.H. ELDER, A. DELL'AQUILA, M. MEZZINA, A. SARASIN and DAPHNE J. OSBORNE
DNA ligase in repair and replication in the embryos of rye, Secale cereale.
Mutation Res., 1987, 181, 61-71.

. C.F.M. MENCK, M.R. JAMES and A. SARASIN
New methods of analysis of radiation mutagenesis in mammalian cells: shuttle virus.
in "Radiation Research" 2, 1987, pp. 550-556. E.M. Fielden, J.F. Fowler, J.H. Hendry & D. Suh, Ed. Taylor & Francis.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.: 1

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. 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 this technique 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:

1) To test and validate a new sub-division key based on chromosome 5.
2) To explore the technique of "reverse staining" in order to characterise late-replicating bands more accurately.
3) To compare the induction of chromosome aberrations in relation to the cell cycle in normal and radiosensitive cells.
4) To investigate and evaluate a new method for quantitative comparison of the replication programmes in normal and treated (or abnormal) cells.

III. Progress achieved:

Methodology

(i) We have now shown that replication bands in human chromosome 5 alone can be used to sub-divide S-phase. Several keys have been tested; the most promising is as follows:-

a { band p13 absent from either homologue - $S_5 1$
  { band p13 present on both - b

b { band q35 absent from either homologue - $S_5 2$
  { band q35 present on both - c

c { band q22 absent from either homologue - $S_5 3$
  { band q22 present on both - d

d { band q34 present as an obvious gap on either homologue - $S_5 4$
  { No q34 gap obvious - e

e { band p14 present as an obvious gap on either homologue - $S_5 5$
  { No p14 gap obvious - $S_5 6$

This key consistently produces 6 fairly even sub-divisions of S-phase in fibroblasts. It has yet to be teted in lymphocytes.

(ii) Reverse staining of replication bands (TB dark, TT pale) is possible, but difficult, and is still not completely routine in our hands. Nevertheless we are satisfied that it will be possible to use it in an experimental situation.

Results

(i) The work with fra-X, referred to in the last report, has been completed, analysed and submitted for publication.

The expression of fra-X can be enhanced by growing cells in low folate conditions. Such conditions also elevate the frequency of conventional chromatid-type aberrations.

Taking advantage of the fact that folate deficiency can be mitigated by the addition of BrdU to the medium, we have analysed the fall in fra-X and aberration frequency in relation to the cell cycle using replication banding, after BrdU addition.

The time taken to reach "control" levels gives a maximal estimate of where, in the cycle, the low folate/fra-site interaction occurs. Such levels were reached well within one cycle; Pre-S cells showed no elevation. The same was true for conventional aberrations.

Sub-phasing provides the facility for unscrambling the scored cells and replacing them in correct developmental sequence. If fra-X is produced at a specific time in the cycle, then expressing frequency per sub-phase should produce an abrupt drop around this time. There was some

evidence for such a crop between SkIII and SkII indicating that fra-X may be an S-dependent phenomenon.

The presence of both fra-X and conventional aberrations (often within the same cell) allowed us to make a very detailed comparative study of the nature of the fra-X aberration. We conclude that the fragile event, which shows a very wide range of expression, does not conform to any known chromatid-type structural change. Nevertheless, there is close parallelism in the fall of both types of change when BrdU is added.

(ii) "Localization by default" Under a previous contract, we investigated the reasons for aberration localization found after alkylating agents. Using Syrian hamster cells we showed that with Sulphur mustard, the localization was an artefact produced by the selective elimination of early S cells, i.e. the scored metaphase sample was heavily biassed in favour of late S cells where aberration localization to late replicating chromatin was inevitable for an S-dependent clastogen. We termed this phenomenon "Localization by default".

One of the most striking localized distributions is that produced by Mitomycin-C in human cells. Having now available a sub-division system for human cells we have tested localization by default for this compound in both fibroblasts and lymphocytes. This work was done in collaboration with two visiting scientists. there was no evidence for selective interphase death of early S cells. All sub-phases were present in the scoring sample and all (including pre-S cells) showed a similar degree of localization.

Since the original "default" was demonstrated with Syrian hamster cells, we feel it necessary to reinvestigate human cells with a simple alkylating agent. Accordingly we have initiated some collaborative work using Nitrogen mustard (a close but more stable relative of Sulphur mustard) with human cells.

(iii) The theoretical aspects of our method for analyzing and comparing replication programmes has now been worked out and is being prepared for publication. We are now in the process of collecting suitable data to serve as tests for its validity and limitations.

IV. Objectives for the next reporting period:

1) To finalize and test the probit/probit method for analyzing and comparing replication programmes.

2) To reinvestigate "localization by default" in human cells.

3) To standardize the reverse staining method in order to examine late-replicating bands in more detail.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Greek Atomic Energy Commission
National Research Center for
Natural Sciences "Demokritos"
Aghia Paraskevi POB 60228
GR - 15310 Athens

Contract no : BI6-E-224-GR

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

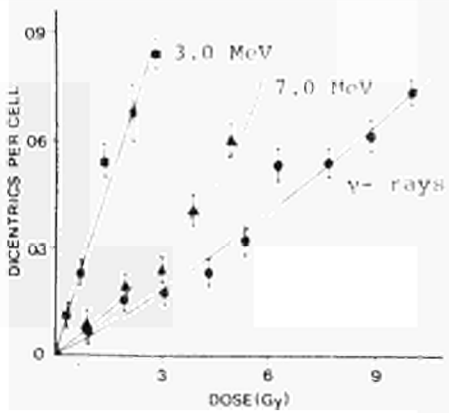
Scientific staff: Dr. A.A. Katsanos
Dr. A. Perris
Mr. P. Pialoglou
Mr. G. Loukakis

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. Background work with human cell lines.

III. Progress achieved: 1. Methodology. Stationary phase culture of V-79 cells were used in order to minimize the deviation of the response of cell population due to differential radiosensitivity of the cells on different cell stages and maximize the percentage of G1 cells in which chromosome type aberrations are induced following exposure to irradiation. Chromosome aberrations were scored in situ on the culture petri dishes following a technique developed in our laboratory (E.C. Sideris et al., 1984, Stain Technology 59:187-192). After exposure to monoenergetic protons or gamma rays and the incubation for 3 h colecmid was added and the incubation was continued for further 18 h so that the cells were arrested at the first post irradiation mitose. The chromosome aberrations were classified according to the IAEA (Technical Report 260, 1986). Human embryonic whole body euploid cell culture is being used in preliminary work with gamma rays so that during the next year part of the work with V-79 cells will be carried out with human embryonic cells. Plateaux phase cells were used in our work on "repair effects" on the survival and aberration induction.

2. Results: Following the estimation in our laboratory of the RBE values from the survival of V-79 cells after exposure to monoenergetic proton irradiation from our Tandem van der Graaff accelerator (Ferris et al., 1986, Intern. J. Rad. Biol. 50:1093-1101) the RBE values from the frequency of radiation induced chromosome aberration was estimated. In the Figure the frequency of dicentrics per cell following exposure to 7.4 and 3.0 MeV protons is presented. From the frequency of chromosome aberrations (dicentrics and acentrics) the coefficients α and β of the dose response curves were determined and compared with those from the two other laboratories which have been published parallel work. These coefficients are given in the Table. When the DNA repair inhibitors 3AB and β-ara-A were used it was found that the ability of cells to survive was decreased while the frequency of induced chromosome aberrations was increased.



3. Discussion: Our results from the chromosome aberration distribution are in agreement taken

RBE values for dicentrics of V-79 Chinese hamster cells

Radiation type	RBE_{α}	RBE_{β}
Cobalt-60 γ-rays	1.00	1.00
7.4 MeV protons	1.1 ± 0,2	1.6 ± 0,2
3.0 MeV protons	6.9 ± 0,7	4.5 ± 0,7

into the account that different end points are measured with our results from survival published previously (ibidem) and with those expected from the theory of dual action (Kellerer and Rossi, 1972, Current Topics Radiat. Res. Quartl. 8:85-158). The coefficients α of the linear quadratic model was increasing with LET. On the other hand no dependence of the RBE values with the dose was found. The difference found on the degree of dispersion between dicentrics and acentrics was attributed to the difference in the repair processes involved in the first case.

IV. Objectives for the next reporting period: Continuation of the work on determining RBE values for low energy monoenergetic protons. Introduction to these studies of human embryonic euploid fibroblasts. Measurements of DSB using the neutral elution method. 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: P. PIALOGLOU and E.G. SIDERIS, 1987, Influence of DNA repair inhibition on the production of chromosome aberrations and cell killing. Proc. 8th Intern. Congress Radiat. Res. Radiation Research, Vol. 1:156.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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:

To obtain blood from a panel of donors, irradiate it, prepare slides from 2-day lymphocyte cultures, encode the slides, distribute them to the participating laboratories and commence our share of the microscope analysis.

III. Progress achieved:

Methods

Blood has been taken from 10 male and 10 female healthy donors aged 20-40y. It has been irradiated acutely with 0,5,30, and 300 mGy of 169 keV ISO wide series X-rays. Standard two-day lymphocyte cultures have been set up and sample slides checked by fluorescence plus Giemsa staining to ensure that there is an acceptably low contamination with 2nd division metaphases. Many replicate slides were produced and encoded. Slides prepared from the material irradiated with 0 and 30 mGy were distributed to all participating laboratories. Each laboratory stained their slides with conventional Giemsa and commenced scoring for aberrations.

Results

The cultures were shown to contain 95% or more first division metaphases. It is intended that each laboratory shall score 500 metaphases per donor per dose. All cells containing 1 or more exchange aberration are being photographed for karyotyping and the x,y stage co-ordinates recorded so that the cells can be re-examined if required.

In the NRPB laboratory approximately 90% of our share of the scoring of the zero and 30 mGy material had been completed by the end of 1987.

Discussion

Until the scoring is completed by each laboratory, decoded, collated and examined statistically we shall not know the results. A decision will then need to be taken on how to proceed with the work. In particular we need to determine whether sufficient donor variability exists to require that the 5 and 300 mGy material also be scored. Karyotyping will also require some collaboration between laboratories as some of the participants are more experienced in this.

IV. Objectives for the next reporting period:

To complete scoring the 0 and 30 mGy material, collate and analyse the results. It is intended to arrange a contractors' meeting at which the data will be discussed and a decision taken on whether it is necessary to score more doses. Material is already prepared at 5 and 300 mGy. The data will be examined for the presence of excessive numbers of multiply damaged cells, containing >1 exchange aberration, which may indicate the existence of a small sub set of extra sensitive cells. The karyotyping results if available will be examined for evidence of in vivo cloning or non-random involvement of chromosomes in exchanges.

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)
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:

The results of the preceding contracts held by this group of participants have been presented at a conference on Low Dose Radiation and the Immune System, May 1987, Frankfurt: D.C. Lloyd, A.A. Edwards, A. Léonard, Gh. 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. (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AI

Contract no: B16-F-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 nondisjunction, and (b) to examine the particular involvement of different chromosomes in nondisjunction.

II. Objectives for the reporting period:

1. To continue analysis of post-implantation embryos after X-irradiation of female mice.
2. To examine pre-implantation stages to determine the fate of embryos with radiation-induced chromosome anomalies.

III. Progress achieved:

1. Methodology

Two experiments have been undertaken to examine radiation-induced aneuploidy in post-implantation embryos: (1) young female mice have been given 4 Gy of acute X-rays. Seven days after treatment they were mated and 7 to 8 day embryos collected subsequently from pregnant females, (2) young female mice which had been induced to ovulate using exogenous gonadotrophic hormones were given an acute X-ray dose of 1 Gy during the immediately preovulatory period. They were mated overnight and 7 to 8 day embryos collected from pregnant females.

Chromosome preparations from different pre-implantation stage embryos (first cleavage division zygotes and morulae/blastocysts) have been made using standard methods.

2. Results

No trisomic post-implantation embryos were found after 4 Gy of acute X-rays to dictyate stage oocytes. Embryos with numerical chromosome mosaicism were observed and were more common in the X-irradiated group (14.5%) compared to controls (6.9%). One of the embryos from the irradiated group was tetraploid.

A somewhat similar pattern was present in the second experiment. After 1 Gy of acute X-rays to immediately preovulatory oocytes no trisomic embryos were recovered. Numerical chromosome mosaicism was higher in the treated group (8.5) compared to controls (2.2%). In addition, a small proportion (4.5%) of the embryos were adjudged to be monosomic; it has not yet been determined whether these embryos have an XO chromosome constitution or tertiary monosomy. The mean number of implantation sites fell from approximately 14.4 in controls to 9.5 in treated females. These implants yielded mean numbers of 12.3 and 5.5 embryos respectively.

3. Discussion

Karyotypic analyses of post-implantation embryos in two experiments have consistently failed to provide evidence of radiation-induced trisomy. To some extent this conclusion is at variance with the expectations from earlier experiments on 1-cell embryos in which X-irradiation was found to increase the proportion of embryos with additional chromosomes (Tease: Mut. Res. 95, 287, 1982; Mut. Res. 151, 159, 1985). The most plausible

explanation for this difference in experimental outcomes is death of these chromosomally anomalous embryos during gestation. Some evidence supporting this interpretation can be gleaned from the data on implantation site and embryo numbers. The reduced number of implantations and of yield of embryos following X-irradiation indicates both pre- and early post-implantation loss of embryos. It is tempting to speculate that embryos with numerical chromosome anomalies contribute substantially to this loss. However, whether such embryonic death is due to radiation-induced numerical chromosome anomalies or to some other aspect of induced genetic damage is uncertain. Experiments are now in progress to assess the fate of embryos with numerical and structural chromosome anomalies during the pre-implantation period of gestation.

IV. Objectives for the next reporting period:

The incidences of X-ray induced chromosomally abnormal embryos will be compared at different stages of pre-implantation development.

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

None

VI. Publications:

None

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Medical Research Council
20 Park Crescent
GB - London W1N 4AL

Contract no.: BI6-E-144-UK

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

Dr. J. Thacker
Cell & Molecular Biology Division
Medical Research Council
Harwell, Didcot
GB - Oxon OX11 ORD

Telephone number: 0235-834393

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.: 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 complete the molecular analysis of DNA and RNA from a series of thioguanine resistant mutants of V79 hamster cells induced by γ-rays, α-particles, ethyl methanesulphonate (EMS) or occurring spontaneously.

(ii) To measure the frequency of mutation in recently-isolated radiosensitive mutants of V79 hamster cells.

III. Progress achieved:

1 Methodology

(i) DNA and RNA was isolated from mutants using conventional methods, for Southern or Northern analysis, respectively, using cloned hprt or aprt probes.

(ii) Mutation to thioguanine-resistance (HPRT enzyme deficiency) was measured using our previously published methods, with particular emphasis on the detection of mutants at low induction frequencies (e.g. use of large cell populations, analysis of the mutant phenotype with autoradiographic techniques).

2 Results

(i) DNA from EMS-induced mutants showing abnormal responses (e.g. high reversion frequency to HPRT-proficiency, undetectable hprt mRNA levels) was analysed on Southern blots, but no altered fragments were found (potentially these could have represented mutants with inserted sequences or gross rearrangements). mRNA from all the HPRT-deficient spontaneous and EMS-induced mutants was run for Northern analysis to check for altered hprt mRNA size. Only one mutant (EMS-induced) had definitely smaller-sized mRNA (from a total of 59 mutants), although a few had such low levels of hprt mRNA that the result was equivocal. Overall mRNA levels were calibrated using a hamster aprt probe.

(ii) A series of experiments with the radiosensitive mutant irs2 has shown that it has a mutant induction frequency per Gy which is similar to the parent V79 line. Experiments with the other radiosensitive mutants isolated in this laboratory, irs1 and irs3, have defined some initial difficulties. irs1 has a tendency to reduced viability when reaching plateau phase and a high spontaneous mutant frequency; selection of subclones and initiation of cultures from a low cell number have probably overcome this. irs3 has a low cloning efficiency, but again screening of a number of subclones has allowed us to identify 2 with much increased efficiencies (50-60%) and one of these has been chosen for further experimentation. In each case, thioguanine concentration survival curves have been established and large scale experiments begun.

3 Discussion

(i) Our completion of the molecular analysis of large numbers of spontaneous and induced mutants has further highlighted the distinction between ionising radiation-induced mutants and spontaneous or chemically-induced mutants at the hprt locus. In the end, we have found only one EMS-induced mutant out of 57 isolated with a major alteration (shorter mRNA but no detectable DNA change), while the majority of γ-ray or α-particle induced mutants carry large alterations to the hprt gene. Spontaneous mutants show a low level (15%) of gross DNA alterations, but among those with unaltered DNA profiles no hprt mRNA changes were observed. Thus, as predicted earlier, the hprt locus has provided an important means of distinguishing the major types of mutation induced by radiation and other agents.

(ii) The finding of a similar X-ray induced mutant frequency for the

mutant irs2 and for its V79 parent, despite their differences in X-ray survival, shows that the two endpoints can be uncoupled. Probably the most simple explanation for this result is that irs2 has an alteration in the repair of a radiation-induced lesion which does not contribute to mutagenesis.

IV. Objectives for the next reporting period:

To establish induced mutant frequencies in the radiosensitive lines irs1 and irs3 and, if these differ from that of the parent V79 line, to isolate and characterise hprt mutants. Development of new molecular techniques to characterise large genetic changes induced by radiation.

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

None.

VI. Publications:

HAMILTON, A.A. and THACKER J. Gene recombination in X-ray sensitive hamster cells. Molecular & Cellular Biology, 7 (1987) 1409-1414.

DEBENHAM, P.G., WEBB, M.B.T., STRETCH, A. and THACKER, J. Examination of vectors with two dominant selectable genes for DNA repair and mutation studies in mammalian cells. Mutation Research, in press.

THACKER, J. Radiation mutagenesis in bacteria and mammalian cells. In 'Radiation Research' (Proceedings 8th International Congress, Edinburgh) (Eds: E.M.Fielden et al) Vol.2, pp 544-549 (1987).

Title of the project no.: 2

The isolation and cellular and molecular characterisation of repair-deficient mammalian cell lines

Head(s) of project: Dr. J. Thacker

Scientific staff: Dr. J. Thacker Dr. P.G. Debenham (to March 1987)

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 independently-isolated radiosensitive mutants and testing their cell survival.

(ii) To extend the application of a molecular assay for the fidelity of DNA double-strand break rejoining to newly-transformed lines from patients with the disorder ataxia-telangiectasia (A-T) and to radiosensitive hamster cell mutants.

(iii) To measure DNA single- and double-strand break repair in new hamster radiosensitive lines using sucrose gradient sedimentation techniques.

(iv) To pursue methods of cloning human repair genes into hamster mutants.

III. Progress achieved:

1 Methodology

(i) Where necessary radiosensitive mutants were genetically marked by transfer of recombinant genes, and then fused in pairs using polyethylene glycol. Hybrid colonies were selected using appropriate media and grown to give sufficient cells for chromosome analysis and to test radiation survival. In addition some hybrids were checked by a novel use of hybridization to human hypervariable probes ('DNA fingerprinting').

(ii) The vector pPMH16 was used as described before. To assay rejoin fidelity in cell-free extracts the plasmid pUC18 was used with assay in tne strain DH5α. Extracts were prepared by isolating nuclei and extracting protein using ammonium sulphate precipitation.

(iii) Radiosensitive hamster lines irs1, irs2 and irs3, as well as the wild type parent V79 and the known double-strand break repair mutant xrs-1, were labelled with ^{3}H-thymidine and irradiated (10 or 20 krad) on ice. After various incubation times at 37°, the initial level and repair of DNA single- and double-strand breaks was estimated using alkaline or neutral sucrose gradient sedimentation respectively.

(iv) Human DNA (30 kb average size) was ligated to a neo cosmid vector and transformed into irs1 cells as described previously. In addition, as part of a collaboration with R. Brown (Beatson Institute), retrovirus-transformed hamster cells were analysed for mutation using Southern blots, as a potential method for rapid cloning of repair genes.

2 Results

(i) Fusion hybrids between lines AT4BI/NE1 and AT5BIVA were found to show no complementation for radiation survival, while hybrids between AT4BI/NE1 and MRC5CV (normal) cells showed either a normal radiation response or a slightly more sensitive-than-normal response. Chromosome counts and DNA fingerprinting indicated that all the A-T/A-T cell hybrids were truly representative of both parents, but this was not true for all the A-T/normal cell hybrids. The A-T/normal cell hybrid with the most representative fingerprint showed the most sensitive survival response, suggesting that the radiation survival phenotype is semi-dominant. A number of new hamster radiosensitive mutants were also fused to a panel of established mutants: BLM-2 (from I.D.Hickson, Newcastle) and VC-4, VE-5 and VG-8 (from M.Z.Zdzienicka, Leiden) complemented the mutants xrs-1, irs1, irs3, XR1, EM7. BLM-2 and VC-4 also complemented irs2 but VE-5 and VG-8 did not. Fusion studies are also underway with the mouse radiosensitive mutant M10 (from K.Sato, Chiba) and some of the panel of hamster mutants.

(ii) Using the pPMH16 vector to assay break rejoin fidelity, the hamster radiosensitive mutant irs3 was found to show the same level of fidelity as the parent V79 line. Attempts were also made to use two newly-transformed A-T lines in the rejoin fidelity assay. AT7BI/CA1 (from A.M.R.Taylor, Birmingham) proved to have an extremely low DNA transformation frequency with pPMH16 and other plasmids as well as a greatly increased sensitivity to the drug selection used in the assay, and work with this line had to be abandoned. AT4BI/NE1 (from L.V.Mayne, Brighton) was transformed with an SV40-based vector carrying one of the genes used in the assay (gpt) : conventional techniques for mutating or eliminating the gpt gene(s) in this A-T line failed, but we now have some promising results using a new

back-selection method against gpt activity. The development of an in vitro assay for rejoin fidelity has centred on the pUC18/DH5α transformation system. To date control experiments investigating restriction and ligation under various conditions have shown the feasibility of using this system.

(iii) Preliminary experiments using a single repair time after irradiation have shown that the radiosensitive hamster mutants irs1, irs2 and irs3 can repair both single and double-strand DNA breaks. We are at present running time-course repair experiments to determine whether these mutants show any kinetic differences in repair from the V79 parent line.

(iv) Further experiments transforming ligated human DNA and marker plasmid into large numbers of cells (2.10^8/experiment) again failed to yield any human DNA transformants of irs1 hamster cells. Southern analysis of transformants known to carry the marker plasmid showed little human DNA was present. Analysis of retrovirally-transformed cells (using the hprt locus as a marker gene to assess the frequency with which the retrovirus mutated known genes)showed that the probability of retroviral insertion mutagenesis was very low.

3 Discussion

(i) The finding of non-complementation for AT4BI and AT5BI is directly contrary to previous complementation data which used the radioresistance of DNA synthesis as the phenotypic measurement. Thus, radiation survival sensitivity and the DNA synthesis defect of A-T cells are separable and may result from mutations in different genes. This finding has important consequences in understanding the wide variety of symptoms of the A-T syndrome. The possibility of a semi-dominant radiosensitivity of A-T/normal cell hybrids has parallels to data of other workers showing intermediate radiation survival capacity in A-T heterozygotes. Among hamster radiosensitive cell mutants we have now extended the number of complementation groups (= genes) to 8, suggesting a complex mammalian cell response to radiation insult. These studies are also of importance to show which mutants carry changes in the same gene (these will not complement in hybrids) to prevent laboratories from duplicating their work where this is thought unnecessary.

(ii) Our results show the difficulty of carrying out the plasmid rejoin assay in some cell types and emphasise the logic of attempting to develop an in vitro assay using cell extracts. It remains to be seen if the latter is practicable.

(iii) Reduced ability to repair DNA strand breaks is common among radiation-sensitive mutants of microorganisms, and has been found in several of the radiosensitive mutants of mammalian cells isolated by others. This is in contrast to the irs mutants we have isolated, none of which appear to have reduced repair of breaks. While this result needs to be consolidated, it suggests we have extended the range of types of radiation lesion which can be examined through mutant analysis. In addition the data for irs1 again suggest that this mutant has strong similarities to human A-T cells.

(iv) In common with results from several other laboratories, the very low levels of uptake of genomic DNA by our mutants suggest that novel techniques are required for repair gene cloning. While the use of retroviral insertion mutagenesis was promising in theory, it does not seem sufficiently frequent in practice to allow for an easy gene cloning protocol.

IV. Objectives for the next reporting period:

To expand studies on fusion hybrids between different A-T lines to show how many genes determine radiosensitivity in this syndrome and to link these genes with changes in specific enzymes. In addition, cell-free extracts of A-T lines and other radiosensitive mutants will be used to establish an in vitro assay for DNA rejoin fidelity. Biochemical measurement of DNA strand break repair in the new hamster radiosensitive mutants will be consolidated, and study of their responses to altered irradiation conditions initiated.

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

Dr. R. Brown
Beatson Institute for Cancer Research
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Bearsden, Glasgow G6 1BD

Dr. P. G. Debenham
Cellmark Diagnostics
Blacklands Way
Abingdon, Oxon OX14 1DY

Dr. D. Chen
Genetics Group
Los Alamos National Laboratory
Los Alamos
New Mexico 87545
U.S.A.

VI. Publications:

1 Publications

DEBENHAM, P.G., WEBB, M.B.T., JONES, N.J. and COX, R. Molecular studies on the nature of the repair defect in ataxia telangiectasia and their implications for cellular radiobiology. Journal of Cell Science, (1987) Suppl. 6, 177-189.

JONES, N.J., COX, R. and THACKER, J. Isolation and cross-sensitivity of X-ray sensitive mutants of V79-4 hamster cells. Mutation Research, 183 (1987) 279-286.

DEBENHAM, P.G., WEBB, M.B.T., STRETCH, A. and THACKER, J. Examination of vectors with two dominant selectable genes for DNA repair and mutation studies in mammalian cells. Mutation Research, in press.

JONES, N.J., COX, R. and THACKER, J. Six complementation groups for ionising radiation sensitivity in Chinese hamster cells. Mutation Research, in press.

DEBENHAM, P.G., JONES, N.J. and WEBB, M.B.T. Vector-mediated DNA double-strand break repair analysis in normal and radiation-sensitive Chinese hamster V79 cells. Mutation Research, in press.

DEBENHAM, P.G. and THACKER, J. The human genetic disorder ataxia telangiectasia: new insights into the basis of radiosensitivity. In 'Radiation Research' (Proceedings 8th International Congress, Edinburgh) (Eds: E.M. Fielden et al) Vol. 2 (1987) pp. 437-442.

2 Short communications

THACKER, J. Repair of X-ray induced DNA damage in mutant mammalian cells. British Journal of Cancer.

JONES, N.J. Cellular and molecular studies of DNA repair in mammalian cells. Ph.D. Thesis, Council for National Academic Awards 1987.

Title of the project no.: 3

The cloning and analysis of radiation repair genes from lower organisms and their introduction into mammalian cells.

Head(s) of project: Dr. P. Debenham, Dr. J. Thacker (to March 1987)

Scientific staff: Dr. P. Debenham (to March 1987)
Dr. J. Thacker
Dr. F. E. Benson (from November 1987)

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:

(i) To clone the engineered recA gene into the inducible mammalian expression vector pMSG and to transfer it into human cells to assess its effect on radiosensitivity.

(ii) To initiate sequencing of the radiation repair gene rorB.

III. Progress achieved:

1 Methodology/Results

(i) A 'trimmed' recA gene was cloned into the inducible pMSG vector and the constructs used to transform E.coli cells. Mini-preps of DNA from transformants were checked on gels and 2 out of 36 were found to have the gene inserted correctly. One of these transformants was grown up to give larger quantities of the construct for mammalian cell transformation. The immortalized human cell lines MRC5CV (radionormal) and AT5BIVA (radiosensitive) were subjected to gene transfer and selected for activity of the gpt gene carried by the pMSG vector. A number of clones grew under selection for each cell line, but on further subculture only 4 clones (all of MRC5CV) survived. Southern analysis of DNA from these clones showed each carries the recA gene region of the construct intact. One of the 4 clones (clone 8.4) on treatment with the inducer dexamethasone shows reduced growth potential (compared to both uninduced clone 8.4 cells and untransformed MRC5CV cells with or without induction).

(ii) Fragments of the rorB gene region were inserted into M13 sequencing vectors and the constructs analysed by gel electrophoresis. Sequencing of these fragments is being carried out at present using the dideoxynucleotide method.

2 Discussion

The integration of the recA gene in a form which can be 'switched on' by an externally-applied inducer is an important step in testing the system of transferring characterised bacterial repair genes into repair-deficient mammalian cells. We should note however that our AT5BIVA transformants died out, possibly suggesting that the gene is 'leaky' and has deleterious effects on the host cell. Some of these aspects can be checked by analysis of the production of recA protein for which there is an antibody (this was part of the reason for using recA in a pilot study).

IV. Objectives for the next reporting period:

To analyse recA-containing clones of human normal and radiosensitive (A-T) cells, assessing the production of recA protein and its effect on various cellular responses. To completely sequence the rorB gene region, 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.

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

None.

VI. Publications:

None.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Contract no.: B16-E-167-NL

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

Prof. Dr. P. van de Putte
Department of Biochemistry
State University of Leiden
Wassenaarseweg 64
NL - 2333 AL Leiden

Telephone number: 071-274768

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. (Mechanism of DNA repair in mammalian cells)

Head(s) of project: Prof.Dr. P. van de Putte

Scientific staff: Dr. C.M.P. Backendorf
Drs. P. Belt
Drs. S. Gibbs (since September 1987)
Dr. A. Kartasova (until May 1987)

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. Analysis of different parameters possibly involved in the induction process.
- Cloning of the XP-A gene. Construction of different cDNA libraries from mRNA enriched in XP-A correcting mRNA.

III. Progress achieved:

1. UV inducible genes in human keratinocytes:

a) Methodology:

Differential screening of cDNA libraries constructed from keratinocytes either irradiated with UV light or unirradiated has been used to identify human genes of which the expression is influenced by UV light. The emphasis has been put on the study of those genes where the relative amount of specific mRNA in the poly A^+ population increases after UV irradiation. During the last contract period most of the work has been focused on two cDNA clones which code for two proteins termed sprI and sprII (spr = small proline rich). Although both proteins are coded for by two different genes they are related as they are composed of similar repetitive elements (see Fig. 1). The expression of both genes has been monitored either by Northern blot analysis or by the use of specific antibodies (in the case of sprI). Repair of pyrimidine dimers in both genes was monitored with T4 endo V and Southern blot hybridization.

b) Results:

UV induced variations in the amount of specific spr mRNA's in the cell were monitored by analysing identical amounts of total cytoplasmic RNA on Northern blots. As 95% of the total RNA is ribosomal RNA originating from cytoplasmic ribosomes, and as the number of ribosomes per cell does not significantly change after UV irradiation, we believe that this method is an easy way to monitor variations in cytoplasmic mRNA concentrations. Although the relative amount of specific mRNA in the poly A^+ RNA population increases for both genes, an absolute increase of specific mRNA in the cell was only found for the sprII gene. The concentration of sprI mRNA in the cytoplasm remained constant. The relative increase of sprI mRNA in the poly A^+ RNA population is due to the fact that the cytoplasmic concentration of most poly A^+ RNA's is decreased after UV irradiation. Indeed we found that the amount of poly A^+ RNA reaches a minimum 6 hours after UV irradiation followed by a recovery between 12 and 24 hours after UV irradiation (the higher the UV dose, the longer the recovery time).

The finding that the cytoplasmic concentration of sprI mRNA is constant after UV irradiation and not decreased as most mRNA's are, is probably not due to enhanced repair in the sprI gene. Indeed preliminary results indicate that the rate of repair of UV induced pyrimidine dimers in sprI (and sprII) is identical to the dimer repair in genes which are decreased after UV irradiation (# 23 and # 77 see last years report).

Induction of the sprII gene occurs between 12 and 24 hours after UV irradiation when total poly A^+ RNA synthesis has recovered. A 5-10 fold increase in sprII mRNA has been measured. Increased levels of sprII RNA are probably produced by de novo synthesis. Indeed we have observed that induced sprII mRNA's are slightly longer than constitutive mRNA's. Preliminary results indicate that the difference in size is due to a difference in the size of the poly A tail. As the size of the poly A tail decreases with time newly synthesized mRNA's are expected to have longer poly A tails.

Tumor promoters such as TPA are powerful modulators of gene expression in mammalian cells and have been shown to induce terminal differentiation in cultured human keratinocytes. In contrast to the results obtained with UV irradiation, TPA increases the cytoplasmic

concentration of both sprI and sprII. The kinetics of induction of both genes are very similar in this case.

In order to analyse whether the expression of spr genes is regulated during terminal differentiation, keratinocytes were cultured in low calcium medium whereafter the concentration of calcium was raised in order to permit terminal differentiation. Cells were analysed at different times after calcium addition. Until now only the expression of sprI has been analysed. Specific antibodies have been raised by using a synthetic peptide corresponding to the C-terminal part of the sprI protein. It has been shown that the amount of sprI protein increases during terminal differentiation. The regulation, however, must be on the translation level as no increase of sprI mRNA is observed during terminal differentiation. The in vitro results are confirmed by in vivo observations. An immunohistochemical analysis of human skin sections showed that sprI expression is very weak in the proliferating basal cell layer but is clearly positive in the granular and spinal layer. No sprI protein is detected in the upper cornified layer.

c) Discussion

The expression of the human genes sprI and sprII has been analysed under different physiological conditions. UV irradiation induces the synthesis of sprII and has no effect on sprI, whereas the majority of cellular poly A^+ RNA's is decreased. From differentiation experiments it seems that sprI is regulated on the translational level. This might as well be the case after UV irradiation. As far as sprII is concerned our results indicate that this gene is regulated at the transcriptional level. A beginning has been made in screening human genomic λ libraries in order to isolate the promoter region of both genes. As both genes are induced by TPA but only one is induced by UV it will be interesting to compare the structure of both promoters and localize the regulatory elements responsible either for UV or TPA regulation.

2. Cloning of the xeroderma pigmentosum group A gene.

a) Methodology

Human genes turn out to be very difficult to clone by direct transfection of mutant cell lines with DNA originating from normal cells. These difficulties are essentially due to two intrinsic properties of human cells: (1) Uptake of only a few copies of foreign DNA (5-10 copies/cell). (2) Integration of mainly short fragments (up to 10 kb) of foreign DNA. Hence, the procedure adapted to clone the human XP-A gene has been chosen in such a way that it circumvents these two problems:

i. Use of cDNA expression vectors which can replicate autonomously human cells: short DNA sequence; no integration problems; high copy number (50-100 copies/cell; selection for enhanced UV survival.

ii. T7 RNA polymerase based cDNA expression vectors: high efficiency synthesis of RNA in vitro; transient correction of mutant cell line by microinjection of RNA; in situ autoradiographic monitoring of unscheduled DNA synthesis (= repair synthesis).

b) Results

cDNA made from poly A^+ RNA enriched in XP-A mRNA (see last years report) was inserted into two different λ vectors:

I. In λEH the cDNA insert is introduced in an oriented fashion 3'

to a T7 RNA polymerase promoter sequence. A library of 300.000 separate plaques was obtained. The length of the cDNA inserts was in the range of 1200-1400 bp which corresponds to the length of the mRNA population used for cDNA synthesis. From these results it appears that more than 80% of the cDNA inserts are full-length. From the original library 8 smaller sublibraries of 6000 plaques each was made and from each of these sublibraries a large scale preparation of DNA was done. Northern blot analysis with known human genes having mRNA's with a length around 1300 bp showed that all 8 sublibraries contained the GADPH gene (abundant mRNA) whereas 1 sublibrary was positive for the HGPRT gene (a rare mRNA species). The different DNA's have been used to synthesize mRNA in vitro using the T7 RNA polymerase system. The experiment was performed either as a run-off transcription by using DNA nicked at a restriction site 3' to the cDNA insert or on unrestricted DNA molecules. In the first case RNA's of ± 1300 nucleotides were obtained as expected whereas in the second case very long transcripts (> 10.000 bp) were observed. Both types of RNA's were capped in vitro and will be microinjected into the nucleus of primary XP-A fibroblasts. Repair synthesis is monitored by in situ autoradiography. These experiments are in progress.

II. In λ ZAP the cDNA is inserted in both orientations into an EcoRI site, flanked by a NotI and a SalI site. This λ vector has the advantage to permit the excision of the cDNA insert with NotI and SalI (both enzymes incise human DNA very infrequently, thus minimizing the chance that the cDNA insert itself is hydrolyzed). In this vector system a library of 30.000 plaques has been obtained. The quality of the inserts was comparable to the λEH library. The 30.000 plaques were amplified and a large scale preparation of DNA was performed. After restriction enzyme hydrolysis the NotI-SalI cDNA insert was cloned into the episomal expression vector pECV-25. Here a library of 300.000 colonies was obtained. Restriction analysis of the pECV library showed that the cDNA inserts had a mean size of 1300 bp, indicating that restriction with NotI and SalI did not significantly fracture the cDNA insert. The pECV library will be used for direct transfection of XP-A immortalized fibroblast and selection of UV resistant colonies.

c) Discussion

During the last contract period two cDNA libraries have been obtained. The λEH library is an excellent library (initial complexity 300.000 plaques) and should contain the XP-A cDNA. The conditions for in vitro RNA synthesis, capping and microinjection have been well worked out, which means that the actual experiment can now be performed. The method is very laborious as if one of the sublibraries will be positive, smaller and smaller sublibraries have to be made in order to end up with one positive λ clone. Hence this approach will claim a large fraction of the research time. The experiments are performed in collaboration with the group of Prof. Bootsma of the Erasmus University Rotterdam.

The λ ZAP/pECV approach is in principle faster. Unfortunately, however, the λ ZAP library obtained is smaller than the λ EH (30.000 initial plaques). Furthermore the cDNA insert has been inserted in a unoriented manner which means that only 50% of XP-A containing clones in the library have the correct orientation for expression of

the XP-A gene. Although it may well be possible that an XP-A complementing cDNA plasmid is present in the library (transfection experiments have been started with this library) it will be important to obtain a larger cDNA library in λZAP (initial complexity of 300-500.000 plaques).

IV. Objectives for the next reporting period:

1. UV regulated genes in human keratinocytes:
   - Cloning of the promoter region of sprI and sprII sequence analysis.
   - Production of antibodies against sprII
   - Study of the influence of mRNA stability and translational control on the expression of spr genes.

2. Cloning of the XP-A gene:
   - Microinjection of in vitro synthesized mRNA's into primary XP-A fibroblast. Monitoring of repair synthesis
   - Construction of a larger λZAP library. Introduction into pECV25. Transfection of immortalized XP-A fibroblasts. UV survival studies.

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

Prof.Dr. D. Bootsma, Dept. of Cellbiology and Genetics, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam

VI. Publications:

- T. Kartasova (1987) Response of human epidermal keratinocytes to UV light. Ph.D. thesis University of Leiden.
- T. Kartasova, B.J.C. Cornelissen, P. Belt and P. van de Putte (1987) Nucl. Acids Res. 15: 5945-5962.
  Effects of UV, 4-NQO and TPA on gene expression in cultured human epidermal keratinocytes.
- T. Kartasova, M. Ponec and P. van de Putte (1988) Exp. Cell Res., in press.
  Induction of Proteins and mRNAs after UV irradiation of human epidermal keratinocytes
- T. Kartasova, G.N.P. van Muijen, H. van Pelt-Heerschap and P. van de Putte (1988) Mol. and Cell. Biol., in press.
  A novel protein in human epidermal keratinocytes. Regulation of its expression during differentiation.
- T. Kartasova and P. van de Putte (1988) Mol. and Cell. Biol., in press.
  Isolation, characterization and UV-stimulated expression of two families of genes encoding polypeptides of related structure in human epidermal keratinocytes.
- E.C. Friedberg, C. Backendorf, J. Burke, A. Collins, L. Grossmann, J.H.J. Hoeijmakers, A.R. Lehman, E. Seeberg, G.P. van den Schans and A.A. van Zeeland (1987) Mutation Res. 184: 67-86.
  Review: Molecular aspects of DNA repair.

VI. Publications (cont.)

Related work:

- B. Kaina, A.A. van Zeeland, C. Backendorf, H.W. Thielmann, and P. van de Putte (1987) Molec. Cell. Biol. 7: 2024-2030.
  Transfer of human genes conferring resistance to methylating mutagens, but not UV irradiation and crosslinking agents, into Chinese hamster ovary cells.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

State University of Leiden
Stationsweg 46
NL - 2300 RA Leiden

Contract no.: B16-E-169-NL

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

Prof. Dr. A.J. van der Eb
Department of Medical Biochemistry
State University of Leiden
Wassenaarseweg 72
NL - 2333 AL 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. The genetic and biochemical basis of radiation sensitivity in human and other mammalian cells in culture.

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 lack of tumors in patients in sunlight-exposed skin aereas. As far as tested, XP cells that did exhibit the usual ER response (ER^+) were derived from patients that were cancer prone. This results suggested that the ER response could be involved in the process of oncogenic transformation. The objective of this project in 1987 was to characterize the ER^+ the and the ER^- cells in more detail, in particular with respect to the stabilization of the p53 cellular tumor antigen after UV-treatment.

III. Progress achieved:

1. Methodology

Cultures of normal human diploid skin fibroblasts and Xeroderma pigmentosum cells were exposed to a proper UV-dose to induce SOS-like functions. A short period after UV-exposure ^{35}S-methionine was added, and the cultures were labeled 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

The time course studies of ER and EM in normal human skin fibroblasts and some XP cells have shown that both phenomena are maximally expressed when cultures are infected 24-48 hrs after UV-irradiation. However, in certain XP-cells of complementation groups A,C,D and G no induction of the ER response was observed, whereas the expression of EM was normal. It was noticed that the latter XP cells ($XPER^-$) were derived from patients which were reportedly free of skin tumors at the time they were described in the literature, whereas the former XP cells ($XPER^+$) were obtained from patients exhibiting skin cancer. This observation suggested that the ER response may possibly be related, directly or indirectly, with oncogenic transformation.

In order to characterize the $XPER^+$ and $XPER^-$ cells, we studied the UV-induced stabilization of the p53 cellular tumor antigen after treatment of growing cells with UV-light. In preliminary time course experiments with normal human skin fibroblasts, it was found that the p53 protein is maximally "induced" (increased in concentration) 3-4 hrs after UV-exposure, which is mainly caused by p53 protein stabilization. The half-life of p53 in UV-exposed cells is about 9.0 hrs. Similar pulse-chase experiments were carried out in $XPER^+$ and $XPER^-$ cells. In $XPER^-$ cells (complementation group A) no stabilization could be detected. Pulse-chase experiments with another $XPER^-$ cell (complementation group C) showed that the p53 protein similarly was not stabilized after UV-treatment. The ER^- phenotype therefore seems to correlate with a lack of the UV-induced transient stabilization of p53. Pulse-chase experiments in normal human cells showed that stabilization of the p53 protein is not only observed after UV-exposure of growing cell cultures but also in resting cell cultures.

3. Discussion

Our results have shown that the ER^- phenotype in XP cells from "tumor-free" patients is correlated with the absence of UV-induced stabilization of the p53 tumor antigen. This suggests p53 stabilization may be one of the factors that can induce certain SOS responses. How these phenomena may be related to cancer induction is completely unknown. We are now investigating whether other proteins are also transiently stabilized after UV-irradiation of ER^+ cells. If this is the case it would indicate that protein degradation pathways are temporarily inhibited after introduction of DNA damage. We will subsequently study protein stabilization in ER^- 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.

J.L.M. van der Lubbe. 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 in press. 1987

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. accepted 1987.

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

1987

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. Wilken Rasmussen
Dr. P. Bach Holm
M.Sc. Xingzhi Wang
Cand.scient. B. Wischmann
Cand.med. J. Glamann

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 (1) the effect of crossing over and chiasma formation on the correction of chromosome pairing in polyploid Bombyx males, (2) The biochemical characterization of the synaptonemal complex, (3) the dosage effects of the Ph gene on synapsis in haploid and hexaploid Triticum aestivum and (4) the short term effect of radiation on meiotic prophase chromosomes.

III. Progress achieved:

Results and discussion.

1. The effect of crossing over on correction of chromosome pairing in tetraploid Bombyx spermatocytes.

Chromosome pairing and synaptonemal complex (SC) formation have been analysed on spread and silver stained chromosome complements from autotetraploid Bombyx spermatocytes (4n = 112).

A mean of 13.3 quadrivalents and 25.1 bivalents was present at zygotene while the number of quadrivalents in pachytene nuclei was reduced to 8.7 and the number of bivalents correspondingly increased to a mean of 37.1. Following the elimination of the SC between pachytene and metaphase I a further reduction occurred in the number of quadrivalents most likely because the extent of chiasma formation was insufficient to preserve all quadrivalents present at pachytene. Entirely random synapsis and SC formation between the four homologues of each chromosome quartet at zygotene would generate a mean of 18.7 quadrivalents (corresponding to 67% of the complement). This number was indeed found in several nuclei. The somewhat smaller mean frequency of 13.3 at 66-95% pairing and 11.1 at 95-99% pairing shows that correction of pairing - converting the quadrivalents into two bivalents - operates during zygotene but is most active towards the end of zygotene and during the zygotene-pachytene transition reducing the frequency of quadrivalents at pachytene to the observed mean of 8.7.

Previous analysis of the formation and fate of quadrivalents in the achiasmatic female Bombyx (Rasmussen, S.W. & P.B. Holm, Carlsberg Res. Commun. 44, 1979) has shown that the number of quadrivalents at early pachytene amounts to 8.4 per nucleus, i.e., nearly the same as in the male. The frequency of quadrivalents at zygotene in the female has not been determined but is most likely similar to the frequency determined for the male. Hence the early events in synapsis up to pachytene appears to follow the same course in both sexes. In the achiasmatic female virtually all quadrivalents present at early pachytene are subsequently transformed into bivalents and by the end of pachytene pairing is exclusively in the form of bivalents.

The comparison between chromosome pairing in male and female autotetraploid Bombyx unequivocally shows that a mechanism exists which has the capacity to optimize pairing in the form of bivalents - a prerequisite for regular disjunction - and that the occurrence of crossing over effectively terminates the correction proces.

2. The biochemical characterization of the SC.

Polyclonal antibodies against the recA protein isolated from E. coli have been raised and assayed for cross reaction with crude extracts of Bombyx testes with negative result. In agreement with this anti-recA antibodies labelled with collodial gold only exhibited unspecific binding to thin sections of lowicryl embedded Bombyx spermatocytes. The search for meiosis specific proteins with recA-like activities has been pursued by the setup of a sensitive assay for the detection of the recA mediated hybrid DNA formation by invasion of ssDNA into homologous dsDNA. The assay is based on

circular ssDNA isolated from phage M13 and the homologous linearized double stranded replicative form. The assay identifies the formation of double stranded circular molecules which move more slowely in an agarose gel than the two precursor molecules. With this assay crude extracts will be tested for recA-like activities.

As a second approach to the identification of proteins with specific functions in meiosis the isolation of DNA topoisomerase II - earlier proposed to be involved in resolution of interlockings (Rasmussen, S.W., Carlsberg Res. Commun. 51, 1986) - has been initiated. The topoisomerase will be isolated from tissure culture cells of Drosophila mel. and its activity assayed by its capacity to convert knotted phage P4 DNA into relaxed circular molecules. The conversion can be precisely monitored by agarose gel electrophoresis. The assay has already identified active topoisomerase II in crude extracts of meiotic nuclei of Bombyx spermatocytes. A soon as antibodies against topoisomerase II are available the antibodies will be tested for cross reactivity with nuclear extracts from Bombyx spermatocytes and the localization of topoisomerase II in the meiotic nuclei investigated with the aid of gold labelled antibodies. This project is carried out in collaboration with Prof. O. Westergaard, University of Aarhus.

3. The dosage effect of the Ph gene on synapsis in the common wheat, Triticum aestivum.

The ultrastructural analyses of synapsis and chiasma formation in wheat has been completed. The material comprises hexaploid wheat nullisomic for chromosome 5B, monosomic for 5B, euploid wheat, wheat where chromosomes 5B have been replaced by one, two or three isochromosomes for the long arm of chromosome 5B, trihaploid wheat with and without 5B and hybrids between hexaploid wheat and rye with and without chromosome 5B or carrying an isochromosome.

The major findings and conclusions on the effect of chromosome 5B on synapsis and chiasma formation in wheat can be summarized as follows:

a) The analysis of euhexaploid wheat has revealed that the exclusive presence of bivalents at metaphase I primarily results from a high stringency of synapsis during zygotene, whereby synaptonemal complexes primarily form between homologues. Most of the few multiple associations formed are subsequently corrected into bivalents before crossing over occurs. As there is virtually no multivalents present at metaphase I irrespective of that a few multiple associations are retained uncorrected through pachytene, it is inferred that crossing over is not permitted to occur between chromosomes of partial homology in euhexaploid wheat.

b) In the absence of chromosome 5B there is in hexaploid wheat a two to three fold increase in the number of lateral components in multiple associations. In wheat monosomic for 5B the number of lateral components in multiple associations is intermediate between that found in nullisomic 5B and euploid wheat while the presence of two isochromosomes for the long arm of 5B appears to result in an even lower frequency of synapsis between chromosomes that are not homologous than seen in euploid wheat. In trihaploid wheat and wheat-rye hybrids, the absence of chromosome 5B results in a two to three fold increase in the number of pairing partner exchanges per lateral component compared to that found in the presence of chromosome 5B.

c) In hexaploid wheat chiasma formation only occurs between homoeologues in the absence of chromosome 5B or if three isochromosomes for the long arm are present, irrespective of that f.ex. in monosomic 5B wheat several multiple associations are retained through the pachytene stage. Trihaploid wheat and wheat-rye hybrids are likewise virtually achiasmatic irrespective of that 35-40% and 40-50% of the complement synapse. Nullisomy for chromosome 5B results in trihaploid wheat in a more than 40 fold increase in the number of chiasmata and a tenfold increase is seen in the wheat-rye hybrids. In the wheat-rye hybrids the replacement of chromosome 5B with an isochromosome for the long arm results in a nearly complete elimination of chiasmata, the mean number being ten times lower than in the euploid hybrids.

d) In hexaploid wheat nullisomic for chromosome 5B wheat synapsis is arrested when 35-40% of the complement has synapsed while the presence of one, two and three copies of the isochromosome results in an arrest of synapsis when 55%, 40% and 25% of the complement is combined with a synaptonemal complex. As monoisosomic 5B wheat differs from euploid wheat, which has an almost complete synapsis, by the absence of the two short arms of chromosomes 5B, it is inferred that the synaptic arrest primarily results from the absence of the short arms of chromosomes 5B. On the assumption that the lack of the short arms of chromosome 5B reduces the extent of synapsis to the same extent in the different genotypes it is apparent that the absence of the long arm or its presence in extra copies additionally reduce the extent of synapsis. In the range from two to four to six copies of the long arm there is an almost linear negative correlation between copy number of the long arm and the degree of synapsis achieved.

It is concluded that the long arm of chromosome 5B has a dual function on meiosis in wheat, its absence resulting in a relaxation of the stringency of synapsis as well as permitting crossing over between partly homologous chromosomes. As similar effects have been described for hybrids between T. aestivum and T. kotschyi, carrying the ph1b mutation of the Ph locus (Gillies, C.B.: Theor. Appl. Genet. 74, 430-438 (1987), it is inferred that Ph may affect a process common to synapsis and crossing over. It is speculated that this process may be heteroduplex formation between single stranded DNA during synapsis at zygotene and during crossing over at pachytene, where in the absence of Ph heteroduplexes may form and persist between DNA of low homology, whereas the presence of Ph either prevents heteroduplexes with a high degree of mismatch from forming or resolves them prematurely.

4. The short term effects of radiation on meiotic chromosomes.

The analysis of the short term effects of radiation on meiotic chromosomes from Bombyx spermatocytes has been continued and a large number of chromosome complements obtained from animals sacrificed every second day up to ten days after irradiation (0-20 Gy, γ-irradiation, 1.17 Me eV, 1.35 Gy/min). The chromosome complements have been spread as described previously (Bojko, M. Ph.D. Thesis, University of Copenhagen, 1985). The subsequent silver staining and EM analysis have just been initiated.

IV. Objectives for the next reporting period:

The objectives for the next reporting period are as outlined in the contract. Special emphasis will be on the further biochemical characterization of the SC and the continuation of the study of the short term effects of radiation on meiotic chromosomes.

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

Prof. O. Westergaard
Dept. of Mol. Biology & Plant Physiol.
University of Aarhus
C.F. Møllers Alle 130
DK-8000 Aarhus C

VI. Publications:

Scientific journals:

1. Rasmussen, S.W.: Chromosome pairing in autotetraploid Bombyx males. Inhibition of multivalent correction by crossing over. Carlsberg Res. Commun. 52, 211-242 (1987).

2. Holm, P.B., X. Wang & B. Wischmann: An ultrastructural analysis of the effect of chromosome 5B on chromosome pairing in allohexaploid wheat. Kew Chromosome Conf. III, in press (1988)

3. Holm, P.B.: Chromosome pairing and synaptonemal complex formation in hexaploid wheat, monosomic for chromosome 5B. Carlsberg Res. Commun. 53, in press (1988)

4. Holm, P.B.: Chromosome pairing and synaptonemal complex formation in hexaploid wheat, nullisomic for chromosome 5B. Carlsberg Res. Commun. 53, in press (1988)

5. Holm, P.B.: Chromosome pairing and synaptonemal complex formation in hexaploid wheat, monoisosomic and diisosomic for the long arm of chromosome 5B. Carlsberg Res. Commun. 53, in press (1988)

6. Wang, X.: Chromosome pairing analysis in haploid wheat by spreading of meiotic nuclei. Carlsberg Res. Commun. 53, in press (1988)

7. Wang, X. & P.B. Holm: Chromosome pairing and synaptonemal complex formation in wheat-rye hybrids. Carlsberg Res. Commun. 53, in press (1988)

8. Holm, P.B. & X. Wang: The effect of chromosome 5B on synapsis and chiasma formation in wheat, Triticum aestivum cv. Chinese Spring Carlsberg Res. Commun 53, in press (1988)

Abstracts:

1. Holm, P.B.: An ultrastructural analysis of the effect of chromosome 5B on chromosome pairing in allohexaploid wheat. Kew Chromosome Conference III, Richmond, England, September 1-4, 1987

2. Wang, X.: Chromosome pairing analysis in haploid wheat by spreading of meiotic nuclei. Kew Chromosome Conference III, Richmond, England, September 1-4, 1987

3. Wischmann, B.: Dosage effect of the long arm of chromosome 5B. Kew Chromosome Conference III, Richmond, England, September 1-4, 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no. BI6-E-170-DK

University of Aarhus
Ndr. Ringgade 1
DK - 8000 Aarhus C

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

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Prof. O.F. Nielsen
Dept. Mol. Biology &
Plant Physics
University of Aarhus
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DK-8000 Aarhus C.

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 carcinogens with the eukaryotic genome and the mechanism of repair. Studies on human and other eukaryotic cell cultures.

Head(s) of project:

O. Westergaard and O.F. Nielsen

Scientific staff.

A.H. Andersen, B.J. Bonven, H. Busk, K. Christensen, C. Herskind, E. Kjeldsen, P.S. Jensen, K. Kristiansen, S.M. Kristensen, T. Stevnsner, B. Sørensen and B. Thomsen

I. Objectives of the project:

The aim of the project is to investigate the effect of ionizing radiation and carcinogens on specific regions of the eukaryotic genome with special reference to the human genome, and to clarify the molecular mechanisms by which DNA damages are repaired.

II Objectives for the reporting period:

The purpose of our investigations has been (i) to study the mechanisms leading to damage of DNA when chromatin is exposed to ionizing radiation and to investigate the effect of scavengers on these processes, (ii) to study the enzymes involved in repair and recombination processes and (iii) to investigate the functional role of sequence specific topoisomerases in repair and recombination.

III. Progress achieved.

Radiation-induced DNA damage measured on a specific eukaryotic gene isolated in its transcriptionally active chromatin form

Transcriptionally active r-chromatin isolated from Tetrahymena provides a unique model system for the investigations of radiation-induced DNA damage since it represents an intermediate between naked DNA and the native chromatin in eukaryotic cells. The chromatin has been irradiated under controlled chemical conditions and the transcriptional activity of the endogenous RNA polymerases on the chromatin has been used to monitor the DNA lesions which block RNA chain elongation. Previous experiments have shown that the OH radical is the major inactivating species, while the H radical give a minor contribution and the hydrated electron can be neglected. The results also showed that secondary radicals may be important for inactivation of transcription when an OH scavenger, e.g. t-butanol, is present. Thus scavengers were found to give strong protection in the presence of oxygen, but only a modest protection was observed under N_2 or N_2O. These data indicate that the attact of secondary radical on DNA under anoxia competes with a decay of these radicals by second order reactions.

When isolated r-chromatin was irradiated in dilute phosphate buffer at neutral pH in the presence of the sulphydrol compound 2-mercaptoethanol, the sulphydryl compound in contrast to t-butanol was found to be more protective against radiation-induced inactivation of transcription under N_2 than under O_2. 2-Mercaptoethanol was found to restore most of the protective effect of t-butanol under N_2. The observed protection may be explained essentially in terms of (i) OH scavenging, (ii) repair of DNA radicals by H-atom transfer from 2-mercaptoethanol under N_2 in competition with fixation of DNA damage under O_2 and (iii) protection against inactivation by secondary t-butanol radicals by H-atom transfer to these radicals. The sensitizing effect of oxygen in the presence of 2-mercaptoethanol is reduced by t-butanol and may be reversed to produce an apparantly protective effect.

The results of the investigations have significant implications for the interpretation of radiation chemical studies on DNA irradiated in aqueous solution or inside intact cells.

The molecular basis for recombination and repair in the eukaryotic genome

From genetic studies with both prokaryotes and eukaryotes it is known that topoisomerases are essential for repair and recombination processes. We have in our studies detected the presence of sequence specific topoisomerases in a number of eukaryotic organisms including man.

Endogenous topoisomerase I introduces site and strand specific single-strand cleavages in the DNA. This enzyme has been purified to homogeneity from a number of organisms and has been shown to have a great binding preference for a specific hexadecameric DNA-sequence motif. Reconstitution experiments with these type I topoisomerases have shown that the preference for the sequence motif is general for eukaryotic organisms, suggesting involvement of the enzyme in a conserved regulatoric process.

Topoisomerase II has also been shown to be sequence specifically associated with the chromatin. The enzyme appears closely associated with topoisomerase I at the regulatoric regions in the chromatin, suggesting that the two enzymes are regulated in a cooperative manner.

Studies on the interaction between topoisomerase II and DNA have shown that in addition to double-strand cleavages in a specific binding region, single-strand cleavage occur in the same sequence as the double-strand cleavages. This observation suggests that topoisomerase II mediated double-strand cleavages occur via two coordinated single-strand cleavages instead of one conserted double-strand cleavage.

A large number of chemotherapeutica are known to interact with type I and II topoisomerases. Since these drugs are known to increase the frequency of sister-chromatid exchange in human cells, it is of special interest to study the interaction between the drugs and topoisomerases. One drug, camptothecin, which inhibits toposiomerase I, has been shown to interact with human type I topoisomerase in such a way, that the sequence specificity of the enzyme is altered and the stability of the complexes formed between topoisomerase I and the DNA is drastically increased.

The effect of drugs on the type I and II topoisomerases are in our studies performed in vitro as well as on a molecular level in vivo in different human cell lines. Also, specific analyses of the function of topoisomerases in vitro and in vivo are being investigated.

IV Objectives for the next reporting period:

During the next period we will concentrate on studying (i) the mechanims that cause damage to DNA when transcriptionally active and inactive chromatin are exposed to ionizing radiation, (ii) the involvement of sequence specific topoisomerases in the processes of recombination and repair, (iii) the mechanism by which chemoterapeutica interact with topoisomerases in human cells and modulate their cellular mode of action.

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

VI. Publications:

Østergaard, E., Brams, P., Westergaard, O. and Nielsen, O.F. Purification and Characterization of an Inducible Mitochondrial DNA polymerase from Tetrahymena thermophila. Biochim. Biophys. Acta 908, 150 (1987).

Christiansen, K., Bonven, B.J. and Westergaard, O. Mapping of Sequence Specific Chromatin Proteins by a Novel Method: Topoisomerase I on Tetrahymena r-Chromatin. J. Mol. Biol. 193, 517 (1987).

Thomsen, B., Mollerup, S., Bonven, B.J., Frank, R., Blöcker, H., Nielsen, O.F. and Westergaard, O. Sequence Specificity of DNA Topoisomerase I in the Presence and Absence of Camptothecin. EMBO J. 6, 1817 (1987).

Busk, H., Thomsen, B., Bonven, B.J., Kjeldsen, E., Nielsen, O.F. and Westergaard, O. Preferential Relaxation of Supercoiled DNA Containing a Hexa decameric Recognition Sequence for Topoisomerase I. Nature 327, 638 (1987).

Herskind, C. and Westergaard, O. Variable Protection by OH Scavengers Against Radiation-Induced Inactivation of Isolated Transcriptionally Active Chromatin: The Influence of Secondary Radicals. Radiat. Res., in press.

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., in press.

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., in press.

Theses.

Thomsen, B. Analysis of the sequence specificity of eukaryotic topoisomerase I by site directed mutagenesis of a hexadecameric recognition motif. Thesis, University of Aarhus.

Busk, H. Preferential base-specific relaxation of plasmids containing a conserved hexadecameric sequence from the intergenic spacer of rDNA from Tetrahymena recognized by topoisomerase I *in vitro*. Thesis, University of Aarhus.

Kristensen, K.H. Specific, distinct DNA sequence elements direct preferential relaxation by topoisomerase I and topoisomerase II. Assessment of the impact of these sequence elements on the expression of a marker gene in mammalian cell cultures. Thesis, University of Aarhus.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Greek Atomic Energy Commission
Nuclear Research Center
"Demokritos"
Aghia Paraskevi Attikis
GR - 153.10 Athens

Contract no BI6-F-206-GR

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

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Laboratory of Biological Dosimetry
Nuclear Research Cent. "Demokritos"
Aghia Paraskevi Attikis
GR - 153.10 Athens

Dr. G.E. Pantelias
Lab of Biological Dosimetry
Nuclear Research 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).

II. Objectives for the reporting period:

A.To develop a C-banding procedure for the accurate analysis of chromosome dicentrics and centric rings in human peripheral blood PCCs, the analysis of which requires unambiguous visualization of their centromeric regions (C-banding), and the quantitation of exposures.

B.To examine whether the fixation and/or repair kinetics of radiation-induced potentially lethal damage is similar to the fixation and/or repair kinetics of radiation-induced chromosome damage in plateau phase Chinese hamster ovary (CHO) cells.

III. Progress achieved:

A.BIOLOGICAL DOSIMETRY

Methodology

To achieve C-banding in PCCs, the following preliminary protocol has been devised: Air dried chromosome preparations are placed in 0.2 MHCl at ambient temperature for 15 min. Excess HCl is removed by gently blotting the slides, which are then treated with 5% barium hydroxide for 5-15 min, depending on the age of the preparations. Slides are briefly immmersed in 0.2 MHCl, rinsed in Sorensen's buffer (pH 6.8), and placed in hot Sorensen's buffer (60 °C) for one hour. They are then stained in 7% Giemsa for 7-10 min.

Results and Discussion

Using the C-banding procedure described above, centric rings and dicentric PCCs can be visualized. Conventional Giemsa staining of PCCs does not allow visualization of chromosome centromeric regions. It is believed that, with further development and standardization, the above protocol may be used for the analysis of ring and dicentrics in PCCs to provide confirmatory evidence of an exposure, especially when blood samples are not available soon after irradiation, and also for quantitative estimates in terms of equivalent whole-body doses.

B.MECHANISMS OF RADIATION ACTION

Methodology

The identification of the sequence of events that lead from DNA damage to chromosome damage and cell death is of particular importance for the elucidation of the action of ionizing radiation in living cells. Experiments were carried out to evaluate the effect of the DNA polymerase inhibitor beta-arabinosyladenine (araA) on radiation-induced damage in CHO cells. When the potentiation of killing of a given araA concentration was studied at the cell survival or at the chromosome level, araA was added to the cells one hour before irradiation. After treatment, cells were analyzed for chromosome damage, as measured by the PCC technique[1], either immediately or after a 24-48h incubation in araA-free conditioned medium. Conditioned medium was obtained from plateau phase cultures and used after filtration to remove floating cells and debris. Cell survival results were measured after a 48h incubation period in araA-free medium, since it was found that such a post-treatment incubation significantly reduced drug toxicity without modifying the drug effect on radiation-induced killing. Plateau phase cells were chilled on ice before irradiation to prevent repair. They were then irradiated on ice using a Siemens therapeutic x-ray machine operated at 250 kV, 15 mA with 2mm Al filter, and then treated with araA for 3h.

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1. The procedure for polyethylene glycol (PEG) mediated cell fusion and premature chromosome condensation induction has been described in detail in Pantelias, G.E., and Maillie, H.D., Somat. Cell Genet. 9:533-547, 1983.

Results

I. Drug toxicity

Incubation of cells with various concentrations of araA for 3h resulted in enhanced cytotoxicity when plating was carried out immediately after treatment. For example, 500 μM araA reduced plating efficiency to 4% of that of untreated controls. However, cell killing diminished when plating was delayed for 24h and was essentially absent at this concentration in cells plated 48h later.

II. Effect of araA on cell survival

An increase in survival was observed in cells plated sometime after irradiation, indicating repair of PLD. The postirradiation (3h) presence of 100 μM araA prevented the increase in survival observed and caused a small potentiation in cell killing. This potentiation in cell killing indicates fixation by araA of radiation-induced PLD normally repaired in cells plated immediately, and was more pronounced after treatment with 500 μM araA.
Cell survival after irradiation as a function of araA concentration rapidly decreased with increasing araA concentration. Concentration of 500 μM araA combined almost maximum potentiation of radiation-induced cell killing with maximum drug toxicity.

III. Effect of araA on radiation-induced chromosome damage

A linear induction of chromosome fragments was observed as a function of the radiation dose with an induction rate of 2 fragments per cell and per Gy. After an exposure of 15 Gy x-rays, a rapid repair was observed in cells incubated post-irradiation in the plateau phase occurring with a half time of one hour. This value is similar to that obtained at the cell survival level for PLD repair. In the presence of 100 μM araA repair was partly inhibited. A complete inhibition of repair was observed in the presence of 500 μM araA during the first 6h.
Experiments were performed also with cells exposed to 8 Gy x-rays and analyzed for residual chromosome damage either immediately after a 3h incubation with various concentrations of araA, or 24h later. An inhibition of chromosome repair with increasing araA dose was observed. Concentrations higher than 600 μM caused a complete inhibition of repair. It is interesting to note that araA concentrations that caused complete inhibition of repair at the chromosome level also caused complete expression of the araA-sensitive sector of PLD.

Discussion

The results suggest that repair and araA-mediated fixation of PLD have their counterparts at the chromosome level as indicated by the similar repair kinetics and inhibition/fixation characteristics obtained for PLD and chromosome damage. Since araA is expected to inhibit the polymerization steps necessary for the completion of DNA repair, it is suggested that DNA polymerization is required for chromosome repair. Among lesions induced by radiation in the DNA, double strand breaks are the lesions more likely leading to chromosome damage. This has been already shown by introducing restriction endonucleases into the cell, or by evaluating the repair kinetics at the DNA and chromosome level (PCC) of ^{125}I decay-induced DNA damage (G.Iliakis, G.Pantelias et al., Int. J. Radiat. Biol., 52, 705-722, 1987).

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

The possible effect on chromosome conformation of DNA repair inhibitors such as cytosine arabinoside, araA, hydroxyurea, and caffeine, will be studied. The role played by such repair inhibitors will be related to the fixation and/or repair kinetics of radiation damage.

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

Dr. G.Iliakis, Laboratory of Experimental Radiation Oncology, Department of Radiation Therapy and Nuclear Medicine, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA

VI. Publications:

Iliakis, G., Pantelias, G.E., and Seaner, R.:
Effect of arabinofuranosyladenine on radiation induced chromosome damage in plateau phase CHO-cells measured by premature chromosome condensation: Implications for repair and fixation of alpha-PLD.
Radiation Research, 1988 (in press).

Related study carried out after approval of the present project but before initiation of its financial support:

Iliakis, G., Pantelias, G.E., Okayasu, R., and Seaner, R.:
125-IdUrd induced chromosome fragments, assayed by premature chromosome condensation, and DNA double strand breaks have similar repair kinetics in G_1-phase CHO-cells.
International Journal of Radiation Biology, 52, 705-722, 1987.

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

1987

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 accidents.

Head(s) of project:

Prof. Agustín Alonso

Scientific staff:

Assist. Prof. Eduardo Gallego

José E. Martín

I. Objectives of the project:

To develop 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:

(Reporting period: 1st July-31th December, 1987)

- Overall design of the model, including each economic submodel and the socio-economic data base.
- Implementation of an atmospheric dispersion model with a flexible meteorological sampling scheme, which could consider the geographical distribution of population and economic centers.
- Starting of emergency countermeasures modelling.

III. Progress achieved:

1.- Methodology.

A calculation model called MECA (Model for assessing the Economic Consequences of Accidents) is being developed. It includes particular models to estimate the direct costs derived from the countermeasures usually assumed to reduce the radiological impact of an accident:

- population evacuation costs,
- contaminated food disposal costs (crops and stock-breeder,
- decontamination costs (urban and rural areas),
- land interdiction costs (urban and rural areas), and
- permanent relocation costs.

A model for the cost of radiation health damage (early and late health effects) is also considered.

The different sub-models will be integrated in a computer code that will need inputs, -such as number of health effects, evacuation, relocation, food disposal, decontamination and interdiction areas-, that could be supplied by most of the modern Accident Consequence Assessment (ACA) codes, together with a socio-economic data base which gathers the site-specific information to be managed by the code. The data-base structure will be compatible with the existing European grid.

2.- Results.

A first application of the model will be based on MACCS Version 1.4 (the Melcor Accident Consequence Code System, recently developed by Sandia National Laboratories for the U.S. Nuclear Regulatory Commission) which is being properly modified to adapt the new assessing capabilities. The outline of the resulting model is shown in the figure.

A flexible sampling scheme of risk dominant meteorological sequences has been introduced on ATMOS (the atmospheric dispersion module of MACCS) in order to allow for user defined spatial intervals for rain or slowdowns in wind speed observations, that can now be adapted to the geographical distribution of towns or industrial areas around a nuclear power plant, instead of the original model with rigid rain and slowdowns distance bands.

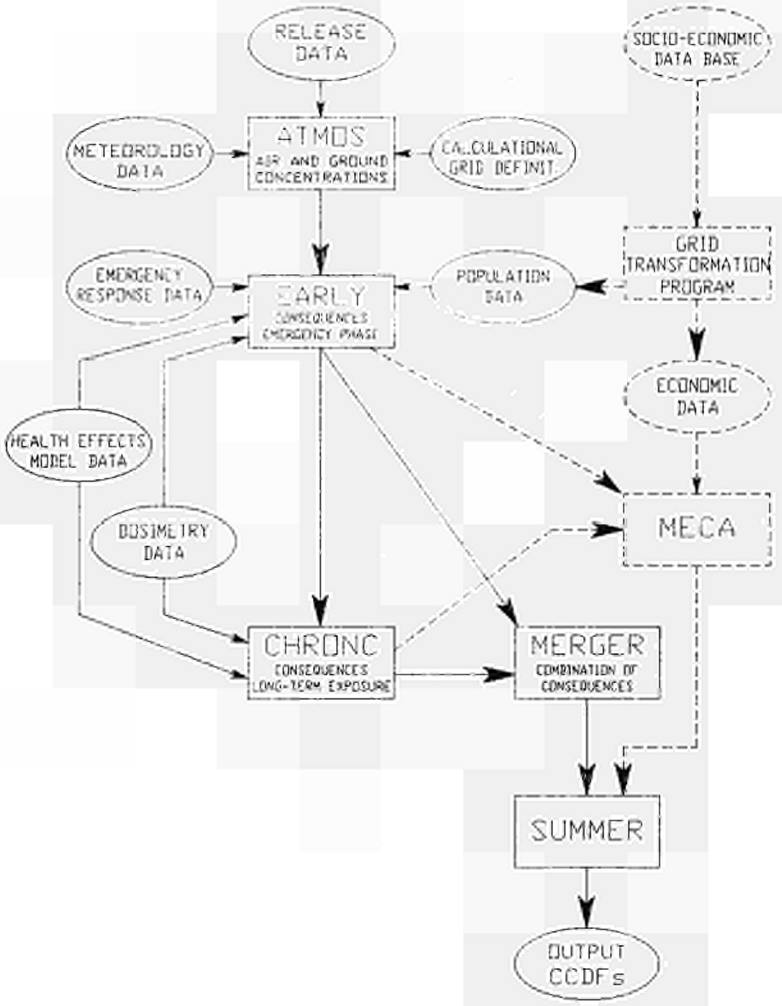
Preliminary observations after this change indicate that it results in a slight increase on high probability-low damage sequences, which leads to a higher mean value of accident consequences.

An extensive change on MACCS data input subroutines has been made to solve incompatibilities between operating systems of different computers.

3.- Discussion.

The main objectives for this period are reached with regard to the overall design of MECA and to the implementation of the meteorological model with flexible sampling scheme.

There has been some delay in the beginning of the modelling of the protective actions during the emergency phase, due to the need of introducing changes on MACCS data input subroutines to have it run in a new computer operating system.



Outline of the joint structure of the MECA-MACCS codes

IV. Objectives for the next reporting period:

* To complete the development of models for the emergency and long-term protective actions.

* To develop each economic submodel to be included in MECA.

* To design the socio-economic data base, including the interface from the European grid to MECA.

* To collect from an Spanish site the socio-economic data needed to perform a site-specific analysis with the new models.

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

VI. Publications:

1.- Alonso, A., Gallego, E., Cost-effectiveness Analysis of Countermeasures using Accident Consequence Assessment Models. In Proc. CEC Workshop on Consequences of an Accidental Contamination of the Urban Environment, (Roskilde, Denmark, 9-12th June, 1987) (To be published in Radiation Protection Dosimetry).

2.- Alonso, A., Gallego, E., Experience on the Evaluation of the Off-site Costs of Reactor Accidents in Spain. CEC Workshop on Radiological Consequences of Chernobyl. (Brussels, Belgium, 3-5th, February, 1987).

3.- Gallego, E., Martín, J.E., Modifications to MACCS 1.4 (Revision 1 for MECA-MACCS). Internal Report CTN-72/87 (Madrid, December 1987).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Contract no : BI6-F-229-E

Centro de Invest. Energéticas
Medioambientales y Technologicas
Division de Medicina
Avenida Complutense, 22
E-28014 Madrid

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.: BI6-F-229-E

- Health effects of chronic exposure to low dose ionizing radiation on workers of the Spanish Energy Institute.

Head(s) of project:

- Dr. F.R. Artalejo.
Subdirección General de Sanidad Ambiental.
Pº del Prado, 18-20 7ª Planta.
E- 28014 MADRID.

Scientific staff:

- Dr. B.S. Fernandez Murias, Dr. A Rebollar, Dr. S. Castaño, Drª. C. Vazquez.

I. Objectives of the project:

a) to measure the mortality of the working population of the Spanish Nuclear Energy Institute (JEN).

b) to measure 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 define the protocols for data collection.

b) to produce adequate software that enables data validation simultaneous with the collection of dosimetric data.

c) to initiate the collection of exposure data from

c_1) dosimetry records from JEN.

c_2) laboral files from JEN.

c_3) clinical histories from the occupational health unit in JEN.

III Progress achieved:

As we programmeed in the approved projet we have attained the aboved stated objetives.

MEDICAL DATA

A number of variables (26 variables that include radiation exposure, tobacco exposure and cancer mortality according to icd 9º) that will be collected from every worker of JEN have been selected. Also, they have been grouped in a common protocol, and those variables that -- are present in each of our data sources (dosimetry records, laboral files and clinical histories) have been identified. It will permit - the validation of information from every worker of JEN.

DOSIMETRIC DATA

As dosimetry record in JEN have changed in format several times -- since 1.956 (startpoint for follow-up) it has been necessary to -- produce a software that minimizes errors in data collection. For - this purpose we have collaborated with the Data Analysis Unit of - JEN that has produced a program, written in FORTRAN, that we have recently checked. Until now we have collected and automatized data from an small number of dosimetry records. Simultaneously, we have obtained and automatized data from 4713 laboral files and 1900 -- clinical histories. So, laboral and clinical data have been obtained for most workers of JEN while dosimetric data (mensual and - - - - bimensual) have been collected for about 2/10 of them. Collection - of exposure data will be completed next year.

IV. Objectives for the next reporting period:

a) to finish the collection of exposure data (dosimetry data).

b) to depurate data.

c) 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).

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

Dra. C. Gorostiza.
DATA ANALYSIS CENTER
CIEMAT.

VI. Publications:

Short communications.

- Health effects of chronic exposure to low close ionizing radiation on workers of the Spanish Energy Institute Proceedings of the II - National Congress on Radioprotection Toledo, Nov. 4-6 1.987 , Spain.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
Forschungsgelande
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:

- Applications to accident consequence submodels;
- Inclusion of further sensitivity measures in SAR;
- Modelling of parameter uncertainty dependences via conditional pdfs in RED (was postponed, new measures of the degree of association were implemented instead);
- Continued development of the package DVA.

III Progress achieved:

1) Methodology

Additional measures of the degree of association which were found to be of interest for the probabilistic modelling of parameter uncertainties were collected. These measures are:

- population quadrant measure q
- population Kendall's τ
- population Spearman's ρ

The first version of DVA was optimized with respect to CPU-time requirements and was added to the set of packages described in the report for 1986.

2) Results

The additional measures mentioned above were implemented in RED. The user is free to select any of the implemented measures he may find suitable to express his state of knowledge about the type and degree of association between uncertain parameters. The empirical equivalents of the additional measures were implemented in SAR together with 2 x 2 contingency tables.

In connection with stepwise regression SAR now also provides PRESS-values upon request. Additionally the user may, via input, force specific parameters into the regression model or exclude them from the model.

ICD has been improved with respect to:

- graphical output of distributions
- handling of data sets from several ICD-sessions for the same application
- generation and graphical output of scatter plots
- arbitrary grouping of several plots in one figure.

First preparations were made for the development of a DRIVER program that is to guide the user through the set of packages according to the steps of an uncertainty analysis. These preparations include the use of panels and the development of a common data interface between ICD and RED.

In the determination of uncertainties in specific conditional probabilities, that could, because of CPU-time limitations, not be obtained via simulation, DVA proved to be a valuable addition to the set of packages.
ICD greatly facilitated the probabilistic modelling of the uncertainty in the parameter "driving time" to be used for given fractions of the population in the model of protective measures of the accident consequence code UFOMOD.

3) Discussion
Advantages and drawbacks of the four alternative design options in RED were pointed out and compared in a contribution to the CEC workshop on "Methods for Assessing the Reliability of Environmental Transfer Model Predictions" in Athens. This contribution particularly discusses the additionally introduced measures of the degree of association. In a further contribution to the above-mentioned workshop the scope and user interface of ICD is explained and illustrated by examples.

A contribution to the CEC workshop on "Recent Advances in Reactor Accident Consequence Assessment" in Rome reports on the probabilistic uncertainty modelling for the parameter "driving time" in UFOMOD. This activity was greatly facilitated by ICD.
The modelling of parameter uncertainty dependences via conditional pdfs has received increased attention and is planned for the next reporting period.
DVA has experienced considerable improvements with respect to the required CPU-time.

IV. Objectives for the next reporting period:

- Further development of DVA particularly with respect to the simultaneous treatment of uncertainties due to stochastic variability and uncertainty due to lack of knowledge;
- ICD will be extended 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 in close cooperation with the institutions mentioned below.

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:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.Ir.P.Berkvens

Scientific staff:

Dr.Ir.P.Berkvens, Dr.H.Vanmarcke, Dr.F.Raes*, Dr.R.Jacobs

I. Objectives of the project:

Systematic study of the physical processes determining the fate of a radon daughter 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 dose. In particular it will be investigated whether the exposure of the public can be expressed in terms of radon concentrations instead of radon daughter concentrations.

II. Objectives for the reporting period:

- In general a steady state situation is assumed in evaluating radon and radon daughter measurements. The uncertainty caused by this assumption is investigated by means of a mathematical model. Priority is given to a number of realistic situations such as smoking cigarettes, opening or closing a door or a window etc. ... .
- Joint measurements were performed with the university of Göttingen to compare our methodologies for determining the unattached fraction in the indoor environment.

* Now at CCR - Ispra

III. Progress achieved:

Methodology

- A Monte Carlo simulator model has been developed to evaluate the uncertainties induced, on the one hand, by assuming constant daughter concentrations and a constant flow rate during sampling and, on the other hand, in calculating the different physical parameters of the radon decay products using the room model.

  The model consists of three parts.

  In the first part a realistic environment is simulated by varying the radon concentration and the parameters of the room model. The attached and unattached daughter concentrations are calculated from the time dependent equations of the room model.

  In the second part a radon daughter measurement is simulated. The number of ^{218}Po and ^{214}Po α counts during sampling and decay are numerically calculated. The air concentrations are computed from these count totals and compared to the steady state concentrations.

  The last part of the mathematical model is the optimization of the deposition rate of the unattached daughters. The fitted value is compared to the input value.

- The behaviour of radon daughters in indoor environments has also been investigated experimentally at the university of Göttingen. Both studies indicated that the fraction of unattached daughters is higher than assumed in earlier studies. The methodologies to determine the unattached fraction however are quite different. They have been compared by means of joint measurements performed in a house with elevated radon concentrations.

Results and discussion

- With the Monte Carlo simulation model it is found that, in general, the uncertainties due to counting statistics and due to the fluctuations in the pump flow rate are small compared to the uncertainties induced by the fluctuations of the parameters of the room model.

  The influence of a sudden change in one or several parameters of the room model has also been investigated. As an example, the effect of closing a door or a window is presented in Figure 1 as a function of the period between closing and sampling. The curves represent the absolute value of the difference between the steady state concentrations

and the measured concentrations, weighted by the steady state concentrations. The difference between the ^{214}Bi concentration and the steady state value is, two hours after the change, still 25%. The fitted deposition rate of the unattached daughters is even 70% higher than the input value. From this Figure and other Figures it was suggested that the large spread in the values for the deposition rate of the unattached daughters reported in the literature is probably in part due to the assumption of a steady state situation.

- The results of the simultaneous radon and radon daughter measurements of Göttingen and Gent broadly agree. The derived parameters however, attachment rate and deposition rate of the unattached daughters, differ significantly. This is due to the different methodologies and to the small unattached concentrations in the house.

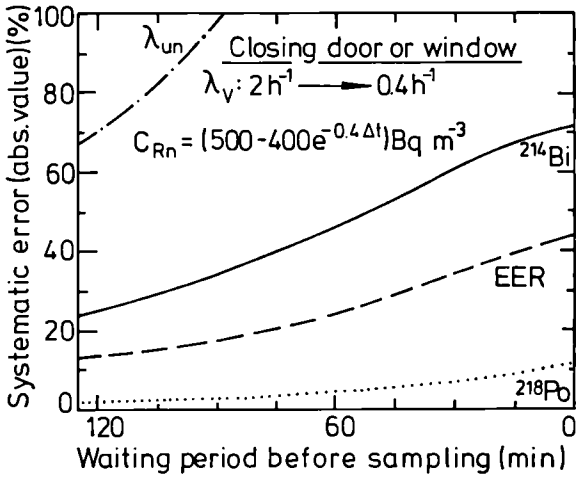


Figure 1. The disturbance of the internal radon-radon daughter equilibrium in a room by closing the doors or the windows. The systematic error is defined as the absolute value of, on the one hand, the difference between the "measured" concentration and the steady state concentration, scaled by the steady state concentration and, on the other hand, the difference between the fitted deposition rate of the unattached daughters and the input value, scaled by the input value. The systematic errors are given as a function of the period between closing the doors or the windows and sampling the daughter concentrations.

IV. Objectives for the next reporting period:

- Continuation and extention of the study of the uncertainties induced by assuming a steady state situation in the indoor environment.
- A detailed investigation of the precise relationship between the dose in the indoor environment and the radon concentration.
- A second intercomparison with the university of Göttingen is planned to further investigate the differences between our methodologies for determining the unattached fraction.

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

- Isotopenlabor der Georg-August-Universität, Göttingen (Dr.J.Porstendörfer, Burckhardtweg 2, Göttingen, F.R.G.)

VI. Publications:

- Vanmarcke H., Janssens A., Raes F., Poffijn A., Berkvens P. and Van Dingenen R. (1987) "The behaviour of radon daughters in the domestic environment : effect on the effective dose equivalent" ACS Symp. Series 331, 301-323.
- Vanmarcke H. (1987) "De bijdrage van het woonmilieu tot de blootstelling aan straling afkomstig van nucliden uit de natuurlijke ^{238}U reeks" Doctoral Thesis. State University of Ghent.
- Vanmarcke H., Berkvens P., Poffijn A., and Raes F. (1987) "Evaluation of uncertainties in assuming a steady state situation in the indoor environment" to be published in Rad.Prot.Dos.
- Vanmarcke H., Reineking A., Porstendörfer J. and Raes F. (1987) "Comparison of two experimental methods to determine the unattached fraction of radon daughters in houses "to be published in Rad.Prot.Dos.

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, Dr.F.Raes*, Dr.Ir.P.Berkvens, Lic.R.Van Dingenen

* now at CCR ISPRA

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 the charge and size of the radon daughters depend on the environmental conditions like humidity, tracer gases and aerosol loading.

II. Objectives for the reporting period:

The deposition rates of aerosol particles and unattached radon daughters have been measured in two different radon chambers and for different levels of turbulence. The experiments were performed on the one side at the laboratory in Ghent and on the other hand at the Bureau of mines in Denver. The general theory of Crump and Seinfeld was used to explain the data. A critical review of the literature supported our findings.

III. Progress achieved:

Methodology

The deposition experiments were carried out in a spherical boro-silicate glass vessel of 0.23 m^3 at the laboratory of Ghent and in the cylindrical stainless steel chamber of 4 m^3 of the Bureau of Mines in Denver U.S.A.. In both cases monodisperse aerosol particles were produced by an electrostatic classifier. The experimental deposition rates were calculated from the decay of the number concentration, which was measured by means of a CCNC. In the Bureau of Mines chamber two fans provided a well defined turbulence while in the Ghent vessel only turbulence by natural convection occurs. The radon daughter experiments in Ghent were done in dry and pure nitrogen such that no growth of the unattached daughters is expected to occur. Following Goldstein and Hopke the diffusion coefficient of unattached ^{218}Po is taken as 0.07 $cm^2\ s^{-1}$. In the Bureau of Mines chamber the size of the unattached radon daughters was measured using a multiple screen method.

The Crump and Seinfeld theory of wall deposition of an aerosol takes into account deposition due to Brownian diffusion, turbulent diffusion and gravitational deposition in a turbulently mixed enclosure. The theory is based on a general expression for eddy diffusion, $De = k_e\ x^n$ with k_e the coefficient of eddy diffusion (a measure of turbulence) and x the distance from the wall. In most studies n = 2 is used. However Friedlander suggests on theoretical grounds n = 3 and Okuyama fits n = 2.7 to his data.

Results and discussion

The deposition rates for monodisperse aerosol particles and for the unattached radon daughters measured in the two chambers are shown in Figure 1. (crosses : Bureau of Mines, circles : Ghent). The curves are calculated using the Crump and Seinfeld theory putting n = 2.61. No good fit could be found for n = 2 and n = 3. The coefficient of eddy diffusion (k_e) was adjusted to the Ghent data while in the case of the Bureau of Mines k_e was calculated from the dimensions and the revolutions per second on the fan. It is seen that the agreement between the experimental and theoretical values of the deposition rates is very good over the whole range of particle sizes and for two totally different degrees of turbulence (k_e).

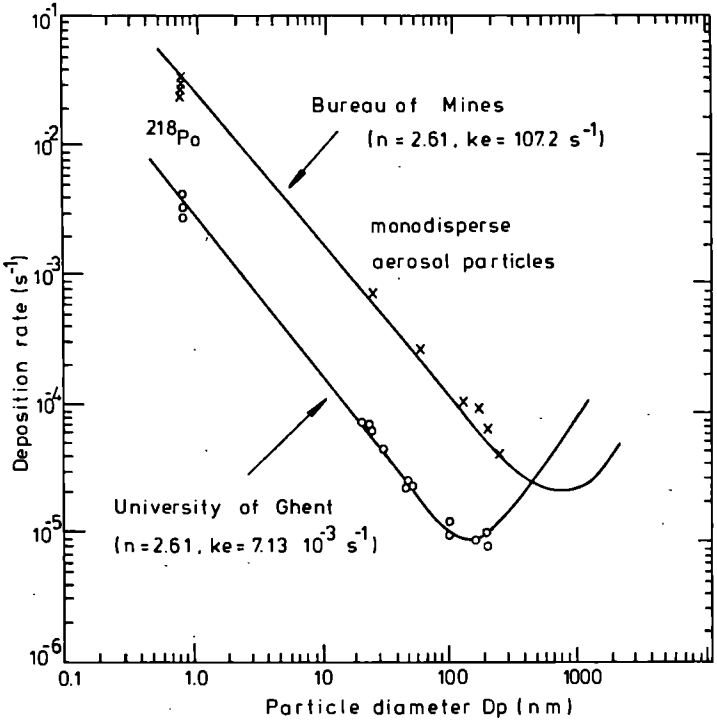


Figure 1. Deposition rate as a function of the particle diameter for two different chambers with different levels of turbulence. The curves are calculated suing the Crump and Seinfeld theory.

IV. Objectives for the next reporting period:

- To check the Crump and Seinfeld theory with n = 2.61. The deposition rate of unattached ^{218}Po will be measured in a 1 m^3 radon chamber as a function of the level of turbulence. The turbulence will be induced by putting heating tape on the bottom of the chamber and by varying the ventilation rate.
- Attempts will be made to apply the Crump and Seinfeld theory to a wider range of environments, including the indoor environment.

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

Bureau of Mines, Denver (Dr.R.F.Holub, Denver Research Center, Denver, CO 80225, USA).

VI. Publications:

- Raes F., Janssens A. and Vanmarcke H. (1987) "A model for size distributions of radon decay products in realistic environments" ACS Symp. Series 331, 324-339
- Holub R.F., Raes F., Van Dingenen R. and Vanmarcke H. (1987) "Deposition of aerosols in different chambers; theory and experiment" to be published in RAd.Prot.Dos.

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.P.Berkvens, Lic.H.Vanmarcke, Dr.F.Raes*, Dr.A.Poffijn

I. Objectives of the project:

The main objective of the project is to inprove 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 the radon exhalation from building materials will be further investigated, using samples provided by the Belgian Building Research Institute (BBRI). A thorough theoretical treatment of the radon transport mechanism will accompany these investigations.

II. Objectives for the reporting period:

The analysis of the nation-wide survey, involving 300 houses has been started. Results from a first specific survey, dealing with houses built on uranium-anomalies were obtained. These studies are done in cooperation with the BBRI and supported by the Institute for Scientific Research in Industry and Agriculture. In the framework of these surveys a semi-automatic counting system has been developed.

In order to obtain a better understanding of the radon source term from building materials and soils an experimental study of radon diffusion through concrete slabs was carried out.

* Now at CCR - Ispra

III. Progress achieved:

Progress Achieved

The nation-wide survey on the radon-concentration in Belgian dwellings has been analysed. In the northern part of Belgium a mean radon concentration of 39.2 Bq/m^3 is found (median value 32.3 Bq/m^3), in the southern part the mean value equals 98.8 Bq/m^3 (median value 59.9 Bq/m^3).

A specific survey has been carried out, dealing with houses that are built on uranium-anomalies. Thus radon-concentrations in 50 houses were measured in Bioul, located on the uranium-anomaly of the Anhée basin. At Denée, a village nearby but not lying on the uranium anomaly, 50 reference houses were selected. For Bioul a mean value for radon concentrations in the living room of 102.8 Bq/m^3 (max 447 Bq/m^3, median 68.3 Bq/m^3) was found, significantly higher than the reference value in Denée (mean 84 Bq/m^3, median 54 Bq/m^3).
For these survey the automatisation of the track-etch detectors read out system was carried out. The apparatus is based on a personal computer (IBM PC/TC), together with a microscope and a video card (e.g. TECMAR VVG-Card) to digitise the picture. The BASIC-program allows now a counting time per foil between 1 and 2 minutes.

As part of a general survey on building materials in Belgium, the exhalation and diffusion coefficient of concrete block of known composition were determined. The diffusion transport was examined by means of two laboratory methods, for a series of different thicknesses.
For a given composition the obtained diffusion coefficient varied over a factor of more than 3 with varying thickness. We concluded that due to the inhomogeneous composition of a material such as concrete the diffusion equation normally used in the pore model is no longer valid. Therefore the values for diffusion lengths for materials such as concrete should be used with great care. If these values are to be incorporated in calculations concerning a limitation policy for radon, an uncertainty of a few 100% should be taken into account.

IV. Objectives for the next reporting period:

Specific radon surveys will be continued.

The study of the radon transport mechanism will be continued, trying theoretical models to be applied for the mechanism in inhomogenous materials such as concrete. The development of a three dimensional code will be continued.

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

VI. Publications:

A.Poffijn, P.Berkvens, H.Vanmarcke ('87)
"On the exhalation and diffusion characteristics of concrete"
to be published in Rad.Prot.Dos.

J.Uyttenhove, J.Paridaens ('87)
"Automatization of a read-out system for radon dosemeters"
to be published in Rad.Prot.Dos.

J.Uyttenhove, R.Lapere ('87)
"Long-term observation of indoor and outdoor radon concentrations"
to be published in Rad.Prot.Dos.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-F-174-I

Univ. degli Studi di Milano
Istit. di Fisica Generale Applicata
Via Festa del Perdono, 7
I-20122 Milano

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- Università di Milano

via Celoria, 16 - 20133 Milano

Scientific staff:
M. Abruzzese, U. Galata, M. Lombardo, M. Magnoni

G.M. Marcazzan, G. Ravasini

M. Decarli, G. Pacchioni

I. Objectives of the project:
Realization of a number of detectors, capable of measurement of radon density in air and giving a continuous record of level density without perturbation of the air itself.
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 neighbouring wells.

III. Progress achieved:

1) Methodology

Different approaches to radon level analysis have been followed: continuous radon detectors, track etch detectors, gamma spectrometer. The track etch detectors are well known; the gamma spectrometer is based on a high purity intrinsec germanium detector.

The continuous detector has been described in 1986 Report. The calibration of the detector has been made with track etch dosimeters and recently our group partecipated to the European Intercalibration Programme in Chilton.

Major results of the intercalibration was to demonstrate the sensitivity of the detector to the aerosol concentration in air: in fact, due to the presence of passive electrostatic field on aluminized mylar, the unattached ions, mainly of ^{218}Po,are strongly attracted by the mylar foil.

When a rigorous measurement is required, we add to the detector a zero field chamber in order to avoid the ions collection.

2) Results

Different places have been explored and mapped.

a) Angera (Lago Maggiore): with the collaboration of the local USSL (Unità Socio Sanitaria Locale) a number of houses have been investigated; at the moment aroud 60.
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 permian phorphyry.
Generally underground water is also in the neighbouring.

The behaviour of radon levels has been followed for two years in the Bertato's house: the strong variations already evidenced in the 1986 Report show a clear autocorrelation with 24 hours period; a strong increase of the levels is observed casually in given weeks.

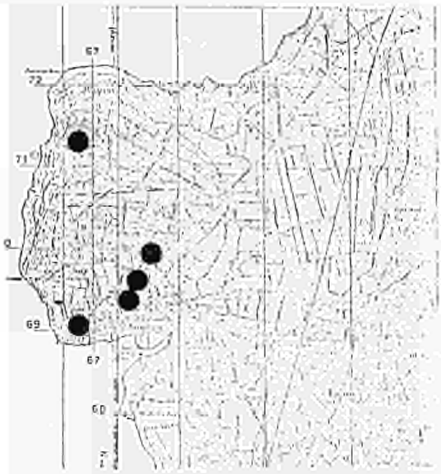


Fig.1: Angera Map

The A.R.L. houses are indicated with black circles

b) Imagna Valley: a strong fracture among different geological structures has been identified in Valle Imagna (Bergamo). 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 (Fig.2). Average radon density in soil along the fracture has been measured by means of track etch detctors; we have the following data:

Altitude around 1000 metres 130-650 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.

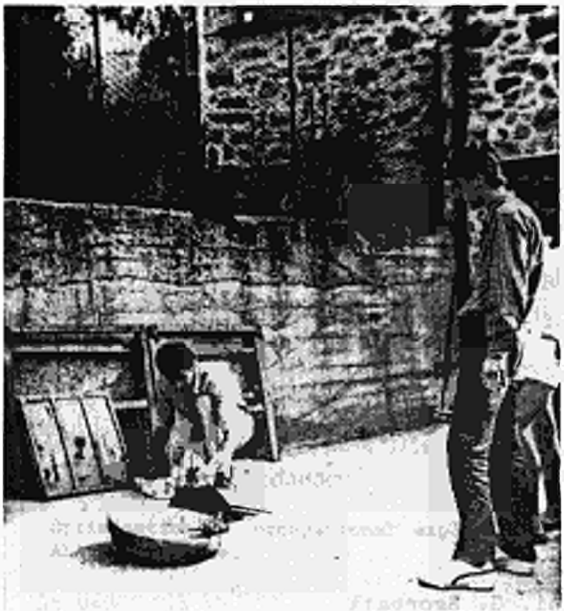


Fig.2
The well at Abruzzese's house. Measurement of radon flux.

c) Castellamonte, near Ivrea in Piedmont: a number of A.R.L. houses has been found: in all houses we have wells and water: the content in water has been measured as 13.000-15.000 Bq/m^3.

Discussions:

In all sites and houses where A.R.L. are observed we have the following aspects:

- presence of fractures
- presence of underground water
- 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.

IV. Objectives for the next reporting period:

Search for the connection of the A.R.L. with water underground and with geological fractures.

Complete the mapping in Angera and in other few sites.

Measurement of radon fluxes from soil surface and underground.

V. 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

VI. Publications:

U. Facchini, G. Ravasini, G. Sgorbati

Misure di radioattività nelle acque minerali e nelle acque sorgive.

Conferenza in Milano, Marzo 88

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

OPTIMIZATION OF OCCUPATIONAL EXPOSURES

Head(s) of project:

LOCHARD J.

Scientific staff:

LOCHARD J., LOMBARD J., MACCIA C., PAGES P.

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 two objectives for 1987 were the following :

- to update the CEPN data bases related to occupational radiation exposure in PWRs.
- to develop a special designed computer program in order to follow occupational exposure associated to large maintenance jobs in an ALARA perspective.

III. Progress achieved:

The compilation of occupational radiation exposure in PWRs has been continued, including at the present time Belgium, Finland, France, Germany, Japan, Sweden, Switzerland and United States. It concerns 110 reactors installed after 1974 June 30th and totalizes about 1050 reactor operating years. The average annual collective dose per production unit between 1975 and 1986 is 0,047 person-rem/GWh for all countries together, but it varies widely according to the country from 0,015 to 0,09 person-rem/GWh. As a function of the number of operating years, this indicator shows a general and regular increase with plants age, excepted in Sweden and United States for which lower values may be noted for recent years. The mean individual doses remain generally fairly constant from year to year at around 200-250 millirem/year /1/.

Concerning the operational dosimetric system, a first phase has been completed concerning the development of an "ALARA predictive plan system" based on a micro computer code to prepare large operations in NPPs. This system is based on the identification of Radiation Analyse Elements (RAEs) which allow to estimate individual and collective doses associated to elementary tasks to be performed within a given operation. These RAEs are combining ambiant dose rates, working times and duration related to a given elementary tasks. Based on the summation of the RAEs, it is possible to predict doses associated to a given operation and to compare scenaries or options in an ALARA perspective /2/. A first application of this system will be performed in 1988 for the preparation of the steam generator replacements to be implemented in the next years in French PWRs.

Apart from theses developments, articles have been published. They concern presentation either of results of previous works performed in the framework of the projects /3/ or general considerations about the practicle implementation of the ALARA principle /4/.

IV. Objectives for the next reporting period:

The next period will be devoted to:

- the updating of the occupational dosimetry data base in PWRs to include the most recent results as well as new parameters allowing a better understanding of individual and collective doses evolutions in NPPs.

- the further development of the "Operational dosimetric system" concerning the follow up of operations and the analysis of past experience based on micro computer codes.

- the performance of ALARA case studies concerning the use of robotics and remote tooling to reduce occupational exposures in nuclear facilities.

V. Other research group(s) collaborating actively on this projects.

None

VI. Publications

Reports:

/1/ LOCHARD J., BENEDITTINI M. - Expositions professionnelles dans les réacteurs à eau pressurisée : comparaison internationale de quelques indicateurs globaux entre 1975 et 1986. CEPN-R-132, Février 1988, Rapport à EDF-SEPTEN.

/2/ LOCHARD J., LEFAURE C. - Caracteristiques et principes de fonctionnement d'un système informatisé de dosimétrie analytique pour les grands chantiers de maintenance. CEPN-R-119, Septembre 1987, Rapport à EDF-DSRE.

Publications:

/3/ LOCHARD J., PAGES P., FAGNANI F., HALLER P., BLAIN M., BREGEON N. Analyse de la radioprotection des premières opérations de microbillage des tubes de générateur de vapeur des tranches 900 MWe. Radioprotection, Vol. 22, n°4, 1987, pp. 357-369.

/4/ SCHNEIDER T, LOCHARD J. - L'introduction du principe "ALARA" dans la prévention des risques sanitaires professionnels. Revue d'Epidémiologie et de Santé Publique, n° 2, 1987, pp. 176-179.

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., OUDIZ A.

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 it 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 1987 were :

- to develop practical guidances for carrying out each step of the procedure which had been defined during the first two years of the contract, for ALARA studies ;
- to apply the ALARA procedure to situations involving radiation exposures of workers and/or the public.

III. Progress achieved:

The work can essentially be divided into two parts : the development of the final report and specific studies concerning the implementation of the ALARA procedure.

1) - Text of the report

The framework for the implementation of the ALARA Procedure has been detailed in the two first years and has since been published (see the two first progress reports). The contents and structure of the final report have been fixed this year and the first drafts of the main text are already performed.

Report structure :

. Executive summary

1. Introduction : What is ALARA ?

   - Historical perspectives ;
   - Concepts underlying ALARA ;
   - The ALARA Procedure ;
   - Levels of decision ;
   - ALARA and practical radiation protection ;
   - Common misconceptions about ALARA.

2. The ALARA Procedure

   - Structuring the problem ;
   - Quantification of factors for each option ;
   - Comparison of options ;
   - Sensitivity analysis ;
   - Presentation and interpretation of results.

3. Practical implementation of ALARA

   - Use of ALARA procedure ;
   - ALARA and strategy decisions ;
   - ALARA and design of installations ;
   - Annexes.

2) - Implementation of the ALARA Procedure

A specific reflexion has been made, in collaboration with the French Atomic Energy Commission (CEA), concerning the implementation of ALARA for high level waste management.
The reports presents the advantages of the ALARA procedure for this fields and the specific adaptations required for this implementation, in order to take into account the problems raised by the long term consideration.
Four case studies, related to different level of decisions, illustrate this various points.

IV Objectives for the next reporting period:

The major objective of 1988 will be the preparation of the draft final report following the structure presented in III.

A study in collaboration with Electricité de France (FdF) concerning the fixation of reference value for the man Sievert is undertaken, as well as a study for low and intermediate level waste management in collaboration with the CEA.

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:

a) - Reports :

- J. Lombard, Ph. Hubert, P. Pagès - Etudes de l'applicabilité des principes de la CIPR à l'analyse de sûreté de stockage géologique des déchets radioactifs. Rapport CEPN n°122. Juillet 1987.

b) - Publications

J. Lombard - Evaluation of radiation detriment. Twenty first midyear topical meeting of the health physics society. Bal Harbour. Florida. December 13-17, 1987.

J. Lombard - Quantitative Decision Aiding Techniques. Twenty first midyear topical meeting of the health physics society Bal Harbour. Florida. December 13-17, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-F-207-F

Centre d'Etude sur l'Eval. de la
Prot. dans le Domaine Nucl., CEPN
B.P. n° 48
F-92263 Fontenay-aux-Roses

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 F. FAGNANI

Scientific staff:

F. Fagnani, Y. Bonvalot, C. Le Galès, P. Hubert, J. Lombard, A. Oudiz, P. Pagès, T. Schneider.

I. Objectives of the project:

- To put into perspective the methodologies aiming at the management of the radiological risk and other industrial risks.
- To demontrate 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:

During the January 1, December 31 period, the main research directions were as follows for the risk management of normal operations :

- Continuation of the establishment of dose-response relationships for chemical carcinogens.
- Analysis of methods for providing rational bases for exposure limits (TLV's).
- Development and application of "systems analysis" in order to determine the "collective exposure" of workers to a carcinogenic substance.

For activities involving risks of accidental nature, the development of risk assessment of risk resulting from hazardous transportation in an important city was set up.

III. Progress achieved:

3.1. Methodological aspects

Common methodological framework

A step by step approach has been set up in the previous period. Performing some case studies has validated globally this approach. Problem analysis, identification of alternative protection options did not raise many theoretical problems and, in particular, the risk assessment tools developped in the radiological field are often readilly applicable (transfer models, etc...). On the other hand, it appears necessary to gain still more experience from case studies in order to give practical rules for selection of the best optimisation tools or clues for the weighting of various risk criteria. The decisionnal framework is more complex in the chemical field, where the fiction of a unique decision-maker cannot be maintained. The general principles of optimisation do apply, but more precise guidelines have to be drawn from further developments of this study. On the other hand, it is thought that this analysis will allow some interesting feedbacks on ALARA practices in the nuclear industry, especially when the problems are not yet fully resolved (managing accident vs routine effects, long term vs immediate, etc...).

Chemical carcinogens management

Dose-response relationships have been derived from a critical analysis of the litterature for Chromiun and Nickel compounds as well has Dioxine.

An analysis of uncertainty in a particular dose-response relationship, (dioxine) has been set up. In this field, a set of animal experiment results has been analysed with one-hit, multihit, probit, logit, and Weibull models. Selection of the model can modify the results by at least three order of magnitude. However, no model apply to all experimental data at the same time. The work is purely descriptive at the present time. The uncertainty analysis has still to be structured, and rules for the management of this uncertainty has to be found.

A methodology for grounding the TLV's for occupational exposures on a common basis, has been developped. The principle is to use the TD_{50} concept proposed by R. Peto, M. Pike and B. Ames for comparing the carcinogenis potenty of the substances (TD_{50} is the dose that would result in a cancer incidence of 50% in a population, after discounting competing mortality causes). The main interest of this approach is that TD_{50} have been made available by R. Peto & al for 776 substances. Assuming an acceptable excess risk of 10^{-4} induced tumors per year (similar to the ICRP objective), TLV can be deduced from simple extrapolation procedures. Comparison with official TLV's for 13 products was performed. The results are within one order of magnitude for MVC, MOCA, Hydrazin, Benzene, Acrylonitrile, but far less consistent for BCME and Carbon Tetrachloride. These results have been published in CEPN report n° 126.

Hazardous material transportation

A computer model TRAMADAN has been set up. It allows for computing the risks linked to an hazardous traffic on a populated area. The results are the overall risk, but also the local risks on any given grid and the structure of the risk, i.e. the probability-consequence curve of the impacts of accidents. It requires data on the products (probability and consequences of failures in traffic accident). They are now available for Chlorine, Ammonia, LPG, motor spirit, Uranium hexafluoride and Plutonium oxyde. The modelized area is a part of the Lyon City (Routes and population density within a 1 km^2 grid).

3.2. Case studies

Occupational risk in chemical industry

The results of the acrylonitrile case study have been published (CEPN report n°126).

The case study on Nickel compounds has been completed. The dose-response relationships can be derived from epidemiological studies in Nickel refineries or from extrapolation of animal data. In the first case, exposure to 1µg m^{-3} (Ni content in compounds) during the working life would result in 0.98 to 3.45 excess cancer cases per 10 000 individuals (lung and nose cancer). Animal extrapolation would result in a risk about 10 times higher. It must be highlighted that many Nickel Compounds can be carcinogenic, but Nickel as such is not proven to be so. It seems reasonable to use the Nickel as a surrogate for exposure to Nickel Compounds, but not to relate carcinogenesis to Nickel itself. In France, the order of magnitude of exposed workers is 10 000. The results are presented in CEPN report n° 130.

The Chromium system has been analysed. The application of system methodology allowed a good insight in the activities resulting in occupational exposure : Metal working, welding and also printing industry. Altogether those sectors represent about 50 000 workers exposed to Chromiun. This methodology resulted in multiplyng by about five the previous estimates. However, the respective importance of hexavalent chromiun, whose carcinogenic potential is pretty established, and trivalent chromiun, for which evidences are weaker, is less well known. Results were published in CEPN report n° 123.

Hazardous material transportation

The comparison of two itineraries for the transit through Lyon has been made on the basis of health risk for residents, road users and on some economic losses due to LPG and motor spirit. The central city itinerary has a risk of .5 expected death per year which seems low for a road traffic. But this risk is linked to rare and severe accidents. All together the expected frequency of accident is one in every sixty year and the average number of deaths for one event is 40. Accidents with less than 10 deaths do not contribute significantly to the overall risk. The other itinerary is a rerouting. It divides the expected number of death by 4 and it reduces also the share of the very severe accidents, that is the catastrophic aspects of the risk. A cost-benefit analysis was performed. When the major hazard aspect of the problem is neglected, a rerouting cannot be proven to be efficient with traditionnal road safety criteria, altough it is not unsound (25 % "deficit"). As soon as some weight is given to major events, even with a factor 2, the selected decision is clearly to propose rerouting (Cf CEPN report n° 129).

IV. Objectives for the next reporting period:

Risk management of normal operations

The methodology for grounding TLV's will be continued. Analysis of the uncertainty and its possible management will be performed. A case study on Benzene is to be completed. The case of management of PCB Transformers will be analysed.

Hazardous material transportation

Risk assessment tools will be developped for detriment other than health effects (i.e. toxic releases in water) but of important economic consequences. Decision aiding tools will be tested on the basis of the results of the Lyons case study. Applicability of the techniques envisaged for dealing with risk aversion in the nuclear field will be tested on this subject.

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

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VI. Publications:

A. Oudiz, C. Le Galès - Approche par filière de la détermination des effectifs professionnels exposés aux composés du Chrome. Rapport CEPN n° 123 - Décembre 1987

A. Oudiz, Y. Bonvalot - Une approche de la détermination des valeurs limites d'exposition aux substances potentiellement cancérogènes. Rapport CEPN n° 126 - Janvier 1988.

C. Le Galès - Estimation quantitative du risque cancérogène associé à certains composés du Nickel. Rapport CEPN n° 130 - Janvier 1988.

J.P. Degrange, P. Hubert, P. Pagès - Estimation régionale du risque associé au transport des matières dangereuses : comparaison d'itinéraires routiers à Lyon. Rapport CEPN n° 129 - Décembre 1987.

A. Oudiz, C. Le Galès - Analyse coût-efficacité de la gestion d'une substance cancérogène : le cas de l'acrylonitrile. Rapport CEPN n° 131 - Décembre 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no · BI6-F-208-P

Laboratorio Nacional de Engenharia
e Tecnologia Industrial, LNETI
DPSR - Azinhaga dos Lameiros à
Estrada do Paço do Luminar
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 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.: 1

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
M.M.R. Teixeira
M.C. Faísca
L. Canelas (DCEA/UNL)
A. Brogueira
M.M. Brito

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:

First phase of indoor radon survey in the regions of Barracão and Urgeiriça.

Gamma spectrometry of soils and tailings. First measurements of radon exhalation from soils of external exposure.

Installation of an anemograph at Barracão.

Preliminary description of the models to be implemented.

III. Progress achieved:

1. Methodology

A survey programme for the determination of radon concentrations in Portuguese dwellings has been established and it started with radon measurements in the granitic region of Beira Alta:

1. in the surroundings of an abandoned radium salts factory (Barracão);
2. nearby the mine and chemical treatment installations of Urgeiriça.

Solid nuclear track detectors were used as passive dosemeters and were exposed for 1-2 months.

Questionaires were distributed with the dosemeters in order to compile relevant data on each house, such as building materials, location of the dosemeter in the house, habits of the inhabitants, ventilation, etc.

Several soil samples from the surveyed regions and some building materials were analysed by Ge(Li) gamma spectrometry.

A detailed comparative study of the technical characteristics and performances of the available anemographs in view of the predicted use of the equipment, was carried out.

In what concerns modelling, some of the models were submitted to criterious trials for the identification of that which might corespond to the needs of the local problems at Barracão and at Urgeiriça.

2. Results

Indoor radon measurements were performed in 33 houses.

The highest values were found at Barracão near the abandoned radium salts factory, with a geometric mean of 1.5×10^3 and a maximum of 3.9×10^3 Bq m^{-3} for the ground-floor of the dwellings, and at Urgeiriça with a geometric mean of 1.4×10^3 and a maximum value of 3.3×10^3 Bq m^{-3} at the offices.

At the town of Guarda, also in a granitic zone, values ranging from 0.15×10^3 to 1.5×10^3 Bq m^{-3} were observed and compared with values for some dwellings from the region of Lisboa, of the order of 20 to 60 Bq m^{-3}.

We also participated in the intercomparison exercise organized by CEC/NRPB.

The anemograph was acquired, but there is some delay in its installation due to delivery date.

Among a large number of models for the simulation of ^{222}Rn in the environment, submitted to analysis, the choice fell on a model which estimates concentrations in air, rates of deposition, intake rates through

different pathways and radiation doses. The dispersion is studied by a modified gaussian plume equation. The installation of this model is on going.

3. Discussion

In general significantly higher values were detected at the ground floor than at the first floor.

The variations observed for the indoor radon concentrations along this short study period do not seem to follow any regular trend according to the month of the year.

The indoor ^{222}Rn concentration observed in the regions under study are, in general, higher than values usually reported.

There is a delay in the installation of the anemograph, as referred, whose implication in the sampling of meteorological data is significant.

Except for this, the proposed objectives were accomplished.

IV. Objectives for the next 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 start of its operation. Sampling and analysis of local meteorologicaldata.
First runs with local data of the model being installed now.

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; Antônio O. Bettencourt
"Preliminary Survey of Indoor Radon Concentrations in Portuguese Houses from High Natural Radioactivity Regions." Presented at the IV International Symp. on the Natural Radiation Environment, CEC-DOE, LNETI, Lisbon 7-12 Dec. 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-F-175-DK

Risø National Laboratory
DK-4000 Roskilde

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 for plume radiation and assessment of factors influencing indoor exposure.

List of projects:

1. Shielding factor calculation for plume radiation.
2. Investigation of factors influencing indoor radon.

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 against gamma radiation from a passing radioactive cloud by indoor residence. Important parameters such as building structure dimensions, 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:

Developement of a mathematical model describing the plume/building geometry. Different building types are composed of cubic boxes making it possible to describe buildings with a relatively complex geometry. Indoor doses are calculated from point kernel integration over the plume volume.

III. Progress achieved:

1. METHODOLOGY

A mathematical model for the description of a plume/building geometry has been developed. Building types are described as a composite of cubic boxes. The thickness of outer walls, inner walls, partitition walls and floors/ceilings can be varied independently of each other. Window apertures are accounted for by the so-called "window-smearing" methods by which an effective outer wall thickness is calculated. This effective thickness is smaller than the actual thickness, reflecting that the indoor dose from a plume both has a "window" component and an "away-from-window" component.

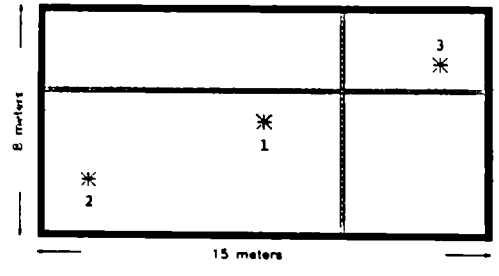
The plume is subdivided in small volumes, and the gamma dose from each is integrated over the significant plume volume. The exponential point attenuation kernel linking dose rate and point-source strength has been used. Attenuation resulting from geometrical spreading with increased distance from the sub-volume as well as exponential attenuation and scattering of the photons in the building materials are taken into consideration.

2. RESULTS

Calculations have been made for a traditional and a modern Danish single-family house as well as for a traditional and a modern concrete 5-story house block. The shielding factors have been calculated as the ratio of indoor dose rate 1 meter above the floor to the outdoor dose rate 1 meter above the ground. The indoor concentration, which contributes to the indoor gamma dose, is assumed to be equal to the outdoor concentration, and the plume size is assumed to be semi-infinite.

Single-family house

The horizontal cross section of the house, the structure data and some of the results are shown below. The three numbers in the figure are the detector positions.



	Traditional		Modern	
Outer wall:	400	kg/m^2	170	kg/m^2
Inner walls:	220	-	100	-
Roof:	200	-	150	-
Window fraction:	25%		25%	

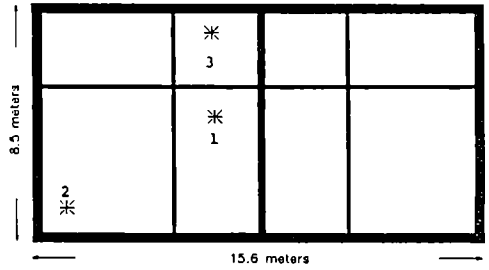
Shielding factors for single-family houses

Photon energy (MeV)	Traditional pos.1	Traditional pos.2	Traditional pos.3	Modern pos.1	Modern pos.2	Modern pos.3
0.12	0.017	0.017	0.011	0.035	0.085	0.042
0.68	0.092	0.11	0.051	0.14	0.24	0.11
2.53	0.16	0.20	0.088	0.24	0.36	0.17

Multistory building

The horizontal cross section of the building, the structure data and some of the results are shown below.

5 story building



	Traditional	Concrete
Outer wall:	595 kg/m^2	410 kg/m^2
Inner walls:	220 -	150 -
Part. walls:	255 -	255 -
Roof:	255 -	425 -
Floors:	170 -	400 -
Window fraction:	25%	25%

Shielding factors for multistory buildings (0.68 MeV)

Story no.	Traditional pos.1	Traditional pos.2	Traditional pos.3	Concrete pos.1	Concrete pos.2	Concrete pos.3
1	0.0023	0.0033	0.0056	0.0011	0.065	0.0057
3	0.0029	0.0025	0.0063	0.0009	0.0032	0.0042
5	0.011	0.0083	0.015	0.0085	0.010	0.013

3. DISCUSSION

The work has been concentrated - in accordance with the objectives - on the development of a mathematical model for different plume/building geometries. The model is now operational and can be used to calculate shielding factors for relevant housing conditions and plume geometries. A parameter study will be carried out in 1988.

IV. Objectives for the next reporting period:

Calculations of shielding factors for different housing conditions with realistic variations of the following parameters:

- building structure data (dimensions and composition)
- plume geometry (position and Pasquill category)
- distance to and dimensions of neighbouring buildings
- photon energy

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

VI. Publications:

None

Title of the project no.: 2

Investigation of factors influencing indoor radon

Head(s) of project:

B. Majborn

Scientific staff:

B. Majborn, A. Sørensen, S.P. Nielsen and L. Bøtter-Jensen

I. Objectives of the project:

To investigate factors influencing the indoor radon concentrations in a group of detached houses, especially the soil conditions at the site of the houses and meteorological factors.

II. Objectives for the reporting period:

- Further measurements of indoor radon concentrations
- Supplementary soil investigations and measurements of radon in soil gas
- Data analysis
- Preparation of final report

III. Progress achieved:

Methodology

The object of the investigations is a cluster of 16 almost identical single-family houses built in 1956/57 as dwellings for Risø employees. The site of the houses forms an area of about 150 m by 300 m. Even though 14 of the houses have identical base constructions (slab on grade), the annual average radon concentrations in these houses vary from about 50 to about 400 Bq/m^3. In view of the varying radon levels, and in view of the relatively high radon concentrations in these houses (i.e. high on a Danish scale), it was decided to use the houses in an investigation of some of the factors that influence indoor radon concentrations.

The investigations comprised:

1. A characterisation of the subsoil at a number of locations on the site.
2. Measurements of the radon concentration in soil gas at a number of locations.
3. Integrating measurements of radon in the living-room and in a bedroom of all the houses on a 2-month basis for two full years (1986 and 1987).
4. Continuous measurements of a number of physical quantities inside and just outside one house for a period of about 2 1/2 months (April-June 1986) when the house was unoccupied. The measured quantities were: The radon concentration in the living room, in a bedroom and in a district-heating duct, the radon exhalation from the soil surface just outside the house, the radon concentration outdoors, the temperature indoors and outdoors, the outdoor atmospheric pressure, and the outdoor-indoor differential pressure. In addition, the air-exchange rate was measured a number of times during the period.
5. Continuous measurements of the radon concentration in 5 of the houses for a period of about 2 1/2 months (March-May 1987).
6. Investigations of the effect of maintaining a slight depression in a district-heating duct which is connected to all the houses.

Results and discussion

1. The soil in the investigated area is mainly composed of moraine clay. However, deposits with a more mixed and permeable composition were found in part of the area. 92 samples of soil from the bore holes showed radon emanations varying from 4 to 17 radon atoms·$kg^{-1}s^{-1}$. Neither the soil samples nor the measurements carried out in the bore holes indicated the presence in the area of any layers with an unusually high radon emanation. Grab samples of soil gas taken at a depth of 50 cm at various locations within the area showed radon concentrations ranging from 4 to 82 kBq/m^3. The results of the soil investigations could not be directly related to the variations of the average indoor radon concentrations. However, the measured radon concentrations in soil gas did demonstrate, that the soil in the area has the potential of acting as a source of relatively high indoor radon levels.
2. The integrating radon measurements showed annual average radon concentrations ranging from 57 to 407 Bq/m^3 in the living-rooms and 38 to 354 Bq/m^3 in the bedrooms. Most of the houses showed a "normal" seasonal variation of the radon concentration with a maximum in the winter and minimum in the summer. A deviating seasonal variation was found in three of the houses. Two of these have a foundation which differs from that of the other houses. In the five houses, where continuous radon measurements were made, the average diurnal variation of the radon concentration showed a maximum in the morning. The hourly data obtained in one unoccupied house during a period of 2 1/2 months showed no or only weak correlations between the radon concentrations and meteorological factors. However, for most of the houses, the seasonal variations of the indoor radon concentrations were strongly correlated with the average indoor-outdoor temperature difference on a 2-month basis. This, and the observed diurnal variations of the indoor radon concentrations, indicate that pressure-difference driven flow of soil gas into the houses is the predominant mechanism of radon entry.
3. It has been demonstrated, that the radon concentration can be strongly reduced in the Risø houses if the district-heating duct is ventilated, so that a slight depression is maintained in the duct.

IV. Objectives for the next reporting period:

This project was terminated per 31.12.1987.
(Project period: 1.1.1986 - 31.12.1987).

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

Department of Electrophysics
The Technical University of Denmark
DK-2800 Lyngby, Denmark

VI. Publications:

Majborn, B., Sørensen, A., Nielsen, S.P. and Bøtter-Jensen, L. An Investigation of Factors Influencing Indoor Radon Concentrations, Risø-M-2689, Risø National Laboratory, 1988.
(To be published).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Contract no. BI6-F-228-UK

Imperial College of Science
Exhibition Road
GB- London SW7 2AZ

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

Scientific staff:
Prof. A.J.H. Goddard, Dr. H.M. ApSimon
Mr. R.J. Cannell, Miss J. MacCurtain

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:
The objectives for the first year of the study included further development work on monodisperse fluorescent aerosols and equipment calibration studies. The initial planned objectives have been modified in that research in a new aerosol test chamber is seen as being an important preliminary to full-scale house measurements and as capable of yielding data in its own right. Commissioning work on the new test chamber has therefore been an important objective.

III. Progress achieved:
Experimental work has involved two aspects; firstly continued development and exploitation of the ultra-violet scanning technique for measuring the spatial patterns of aerosol deposition and, secondly, commissioning work on the aerosol test chamber.

Data produced using the U-V scanning technique should help to reduce uncertainties in parameters involved in modelling sheltering properties of buildings. The U-V technique provides a sensitive, non-invasive technique for areas of interest, for example walls and floors. The imaging system has now been enhanced in that it employs an image intensifier, narrow band-pass filters and improved CCTV. This improved system has been tested in conjunction with monodisperse silica particles of size ranges of interest, which had been tagged with fluorescent material. Tests had included confirming a linearity of response with dust loading on a surface, over the range of loadings of interest.

Experimental work on mechanical transport of dust on flooring surfaces has continued. Measurements of dust transport through the action of footwear has covered 0.1, 1.0 and 3μm diameter particles for a range of floor coverings, footwear types and contaminated surface loadings.

During the reporting period, the aerosol test chamber has been erected and is now being commissioned. This is a 2m aluminum cube with aerosol generator, fan and ducting and with an inspection door to allow access for the UV imaging system. The chamber has been equipped with a low-speed spherical, flow direction independent, anemometer and with humidity and temperature probes.

Following completion of commissioning in February 1988, work will begin on studies of deposition on a variety of indoor surfaces, oriented both horizontally and vertically, initially with 3μ particles, later extended to 1.5μ and possibly to 0.1μ . Planning will be undertaken for the adaption of the

chamber for resuspension and for particle infiltration studies. The next stage will be the development of the UV imaging system together with other methods, in an actual dwelling. The equipment is being modified to improve mobility; its use within dwellings should permit investigation of ingress, deposition and resuspension.

The diagram shows illustrative results for the mechanical transport over concrete, of particulate material from a contaminated area, by footwear having a range of contact areas. Material was transferred by one transit only, from an area contaminated initially with 250 μg cm^{-3} of 3 μm particles.

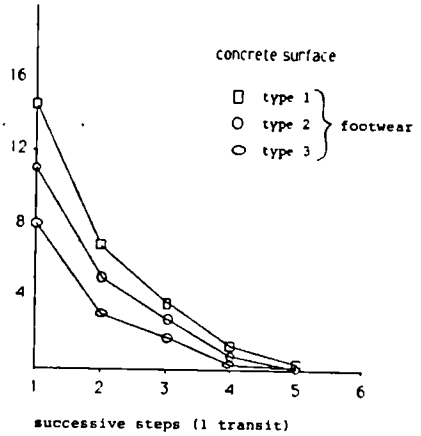


Figure 1. Contamination of concrete surface

IV. Objectives for the next 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.

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

-- Riso National Laboratory
Denmark (Jorn Roed)

-- Environmental and Medical Sciences Division
AERE Harwell, U.K. (Dr. K. Nicholson)

VI. Publications:

1. Cannell, R.J., ApSimon, H.M. and Goddard, A.J.H. The Tracking and Measurement of the Ingress of Particulate Matter into Urban Dwellings in PROC. AEROSOL SOCIETY CONFERENCE, March 1987, Loughborough, U.K.

2. Cannell, R.J., Goddard, A.J.H. and ApSimon, H.M. Contamination of Dwellings by Particulate Matter: Ingress and Distribution within the Dwelling. RAD. PROT. DOSIM., in press.

3. Cannell, R.J., Goddard, A.J.H. and ApSimon, H.M. Particle Transport in Urban Dwellings. PROC. CEC. Workshop on Recent Advances in Reactor Accident Consequence Assessment. January 1988, Rome.

RADIATION PROTECTION PROGRAMME
Progress Report

1987

Contractor: Contract no.: BI6-F-108-UK

Imperial College of Science
and Technology
Exhibition Road
GB- London SW7 2AZ

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.: Pathways and systems pertaining to the urban environment

Head(s) of project: Dr. H.M. AbSimon Prof. A.J.H. Goddard
Mechanical Engineering Department
Imperial College, London SW7 2AZ

Scientific staff: Mr. K.L. Simms Mr. R.J. Cannell

I. Objectives of the project:

The first part of this 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 recent Chernobyl accident, which has been used to evaluate the methods developed. The second aspect was originally concerned with making use of existing information, on the behaviour of particulate activity in dwellings, to evaluate exposure due to deposited activity. The inadequacy of this 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:

The objectives for the final phase of the first part of the study were to apply the RAINPATCH computer model, developed to estimate wet deposition using detailed precipitation data from weather radar, to a large number of hypothetical releases and examine the implication of the results for PRA. The second part of the project had the objectives of development of the DIADEM particle scanning technique into a reliable tool and collaboration in joint experiments relating to the urban environment

III. Progress achieved:

To provide good spatial and temporal resolution of rainfall in different types of meteorological situation, an extensive data base of weather radar observations was established. Wind-field data was also collected to calculate trajectories. A computer model, RAINPATCH, has been developed to simulate dispersal of a radioactive release as a puff, or a sequence of puffs, and to estimate the wet deposition. Each puff is differentially scavenged according to the rainfall encountered, with some turbulent remixing.

Chernobyl provided an opportunity to test the RAINPATCH model, which proved a useful tool in analysing the pattern of deposition of Cs over England and Wales. The model has also been applied to 300 hypothetical releases, and the results compared with results generated by artifically assuming that meteorological conditions are always the same as at the source (as in most current PRA assessments). Although there were vast differences in individual situations due to real meteorology as opposed to source meteorology, statistically the overall differences were much less than expected. The source meteorology tended to be more pessimistic as distances increased.

In earlier phases of the project important differences were pointed out in wet deposition estimates using a puff model instead of a segmented plume model. However, for statistical applications in PRA, when using a puff model, no significant difference was found in wet deposition out to 300 km from the source, allowing for spatial as well as temporal variations in meteorology. This gives more confidence in PRA analysis based on puff models.

The potential usefulness of the RAINPATCH techniques within emergency procedures has been demonstrated. Development of more detailed 2-D storm models has been initiated following this work in order to study mechanisms which may concentrate

or enhance deposition locally.

In respect of the behaviour of particulate material in dwellings, the DIADEM UV scanning technique for measuring particle deposition patterns on indoor surfaces has been developed fully, with the aid of image intensification, narrow band-pass filters and improved CCTV. A range of calibration studies has been completed and the technique is now being exploited, under contract BI6-F-228-UK, to generate data for radiological assessment in respect of indoor exposure.

Collaborative work has been undertaken at Riso, with the National Laboratory acting as the lead organisation. Beryllium-7 was used as a tracer to study the deposition of small particles on surfaces in a furnished and an unfurnished room of a single-family dwelling, and on the internal surfaces of the entire house.

IV. Objectives for the next reporting period:

Further work on the effect of the physical and chemcial processes on wet deposition is planned, aided also by other sources of support within the U.K. In respect of particulate transport within dwellings, a computer model will be developed which will represent a range of occupant dose pathways within dwellings.

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

In developing the first part of this study for real time applications we shall be collaborating with the U.K. Meteorological Office, Bracknell, Berks., U.K.

Collaboration in exposure pathways within dwellings will be maintained with Riso National Laboratory and with AERE Harwell.

VI. Publications:

1. ApSimon, H.M. and Simms, K.L. Estimating the effects of rain and snow on potential reactor accident consequences. Nuclear Energy. 1986 25. No. 4. Aug. p. 235-42.

2. ApSimon, H.M., Manning, P. and Simms, K. Facts and Fallacies in wet deposition modelling. Proc. British Ecological Society. No. 6. 1987. p. 113-123.

3. ApSimon, H.M. and Simms, K.L. Initial assessment of deposition from Chernobyl over England and Wales. Accepted for publication in Atmospheric Environment.

4. Roed, J. (Riso) and Cannell, R.J. (ICST), The deposition of beryllium-7 marked particles on surfaces in unfurnished and furnished rooms. Proc. CEC Workshop on Recent Advances in Reactor Accident Consequence Assessment. January 1988, Rome.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

Dir. P. Govaerts
Dept.Stralingskontrole en Veiligheid
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.

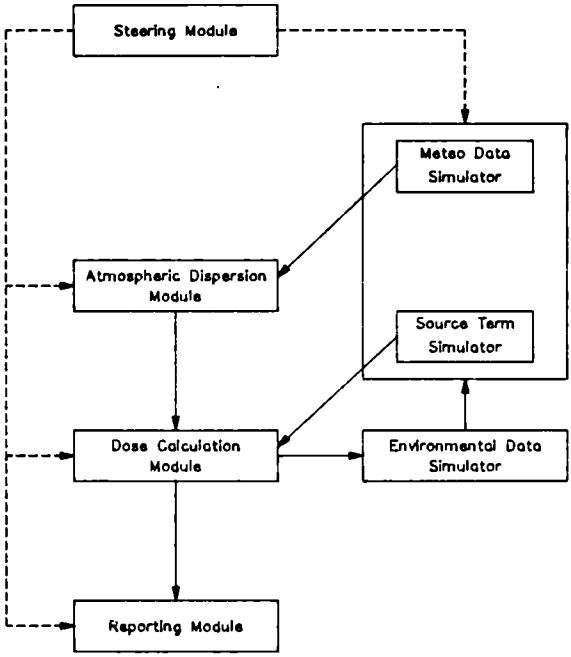
II. Objectives for the reporting period:

- Test of the source term simulator.
- Replacement of the cloudshine model by a more elaborated version.
- Incorporation of foodchain evaluation for the main radionuclides and pathways.

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 figure.



2. RESULTS

The activities in this project were limited to the testing of modules, developed previously.

The atmospheric dispersion module was intensively used for the simulation of tracer experiment and the testing of feedback procedures, mentioned by the progress report of project 2.

The atmospheric dispersion module is extended by an option to consider topography, by assuming a deformation of flowlines in function of stability. This method seems relatively succesfull, as far as the horizontal projection of the flow remains parallel to the wind direction observed at the point of release. The applicability of this method in a real-time model has been tested using reported results of complex terrain tracer experiments.

The straightline bi-gaussian version of the atmospheric dispersion module is compared with the tri-gaussian version for some experiments.
It seems that for average realistic situations, the tri-gaussian version does not increase the model performance.
The dynamic foodchain model of the SCK/CEN (DOSDIM), which is also intended to be modified in order to be coupled to the real-time accident management package, is tested by simulating time-dependent concentrations in food for several locations, using the Chernobyl impact data.
This allowed to build up some in-sights in the robustness of generic parameters values, considered by the model.

DISCUSSION

A part of the existing modules of the real-time model has been tested during the reporting period.
The coordinated test of the source term simulator and the replacement of the cloudshine model by a more elaborated version is postponed to the next period.
The foodchain model is tested, but the coupling of the module with the real-time model has still to be made users friendly.

IV. Objectives for the next reporting period:

- Test of the source term simulator.
- Coupling of existing modules to a more users friendly system.

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

VI. Publications:

P. GOVAERTS, A. SOHIER, G. FIEUW, - "Computer assistance in case of large radioactive releases to the atmosphere", Seminar on the application of Computer technology to radiation protection, IAEA-SR-136/67, Bled, Yugoslavia, June 1987.

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:

- Analysis of the feasibility of the optimisation technique to more complex situations.
- Review of radiological survey methods with respect to the feasibility of the optimisation process.

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

2.1. Simulation of environmental survey results.

A data-base is built up with reported results of tracer experiments for various meteorological conditions, release heights and terrain complexities (flat and hilly). Those experiments relate only to concentration measurements.

Values for deposited activity and related instrument responses can be made artificially or derived from measured concentrations applying deposition parameters with a controlled scatter around a mean value.

2.2. Development of an optimisation technique.

Two approaches are compared as shown by figure 1.

* The mathematical optimisation of a statistical expression, related to the differences between measured and calculated values, using input parameters as variables (approach A).
* The spatial regression of observed data, assuming some dispersion model. The first estimate of the model parameters can be compared with the derived regression parameters and eventually corrected, regarding the differences (approach B).

The former approach is easier to handle by traditional models, but includes the risk of generating physically unlike combinations of parameters which fit mathematically well. The latter is physically better controlled but can only be applied if enough data are available to make regressions.

The first technique was evaluated on the hand of tracer experiments. The realisation of an automatic feedback system asks for a lot of options :

- a hierarchy of parameters, selected according sensitivity to calculated results and degree of uncertainty of the default parameter value;
- a window for each parameter value, and a step by which the parameter has to be modified for each optimisation loop;
- an acceptance criterion;
- a rejection criterion.

Those options are still controlled by the research team. The system can only be considered as feasible if those options can be fixed or selected by the user of a real-time assessment system.

Tests were related mainly with the influence of those options to the stability of the optimisation.

2.3. The environmental survey program.

A typical emergency survey program of nuclear power plant was analysed. This study should lead to the generation of information sets, which are felt to be realistic for the data, available in function of time to the emergency team.

Some theoretical requirements to the survey program are defined to make it more suitable for the model parameters optimisation process.

Figure 2 shows the number of measurements needed to predict at a given level of confidence a parameter, which is proportional to the impact, e.g. the source term, in function of the median error of the applied transfertmodel.

3. DISCUSSION

The project is progressing as proposed by the previous report.

Fig. 1 : General scheme of the optimisation system.

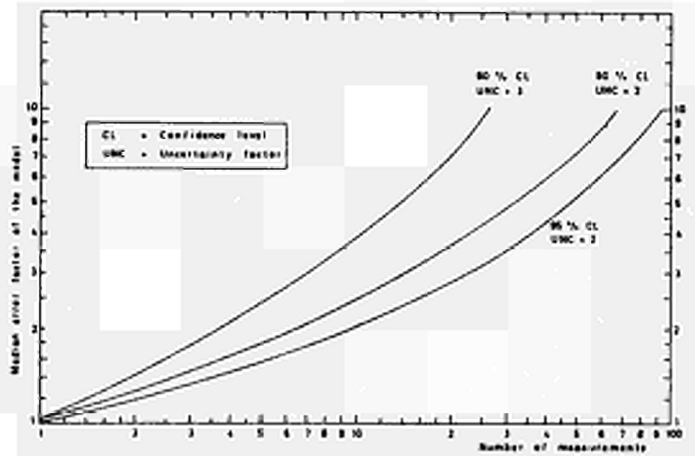


Fig. 2 : Number of measurements required to obtain a given degree of confidence.

IV. Objectives for the next reporting period:

- Testing of the two optimisation approaches 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.

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

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:

- Reporting of raw data for scenario selection.
- Elaboration of a logical scheme for scenario selection.
- Development of a computer assisted system for scenario selection.
- Preparation 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.

Because of the dependence of this project on the outcome of the first project, those tasks can for the moment only be partially performed.

It is decided to perform the points 1 to 3 by a computer assisted method on P.C.

2. RESULTS

The set of raw data was extended for several heights of release, release duration and probability levels. The data are available as doses to organs for specific exposure pathways, due to individual radionuclides and physico-chemical groups of radionuclides.

The results are accessible on P.C. The publication of a hard copy of the basic data set is still under preparation.

3. DISCUSSION

The elaboration of a computer assisted system for scenario selection, based on the needs for training is delayed.

Main emphasis was still put on the elaboration and testing of the set of raw data.

IV. Objectives for the next 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.

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

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-F-131-UK

United Kingdom Atomic Energy
Authority, UKAEA
11 Charles II Street
GB- London SW1 4QP

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: (Dec. 1986 - Dec. 1987)

a) To complete the investigation of the fundamentals of puff-trajectory modelling and to write a report of the work;

b) To examine the potential impact of deposition in foggy conditions (sometimes termed 'occult' deposition) on probabilistic consequence assessment and write a report on this;

c) To take up again the investigation of the dry deposition of large particles for which gravitational settling is important, in particular examining what can be gained from using Lagrangian-particle techniques.

III. Progress achieved:

Methodology

a. An investigation has been carried out into the justification for, and the benefits of, using the puff-trajectory model to achieve a more realistic representation of the time-integrated concentration field from a specific long-duration release. The implications of using the technique in the context of probabilistic consequence assessment have been examined.

b. Foggy conditions have not been explicitly dealt with in probabilistic consequence assessment to date, and an examination of the potential impact of this omission has been performed. This focusses on the influence on deposition levels of a fraction of the released material becoming incorporated into fog droplets and thereby being subject to a considerably enhanced deposition velocity. Both theoretical and experimental information are utilized. The investigation considers incorporation both as a result of formation of droplets on containment particles and via attachment to existing droplets. A variety of fog types occur in practice, so three representative scenarios are chosen to span the range of situations of interest. Example calculations are performed to illustrate the potential impact of increased close-in deposition and to determine the magnitude of increased attenuation in the quantity of contaminant remaining airborne. Fog frequency data are utilized to further clarify the impact on probabilistic calculations.

c. An earlier study contract (1225-83-9L/V) with the Radiation Protection Programme went some way towards clarifying the ranges of applicability of a number of simple extensions to the Gaussion model to allow for the dry deposition of larger particles (ie. particles with appreciable gravitational settling velocity). The 'benchmark' model against which the simpler approaches were compared was based on eddy-diffusivity theory. However, this is known not to be adequate when significant deposition occurs close to the release point, and a recommendation for future work was that a better 'benchmark' be used for this case, possibly one based on Lagrangian-particle techniques. This suggestion has now been taken up. As a preliminary, the question of how generally to incorporate a realistic deposition boundary condition into the Lagrangian-particle model is being addressed. Techniques are being tested out first on the homogeneous-boundary-layer case.

Results and Discussion

a. The alternative way of looking at the puff-trajectory approach has led to answers to a number of questions such as : what is the basis for using ensemble-average 'spread' parameters (σ values) in the simulation of particular realisations of the concentration field and to what sampling time should these σ values correspond? The interpretation establishes a correspondence between the omission of wind-field fluctuations with period below a given value, T, and the spatial resolution of the time-integrated-concentration field. Quantitatively, the spatial smoothing length of the TIC estimate is related to the width of the ensemble of potential deviations of serially released particles from their trajectory positions, arising from fluctuations with timescale less than T. The investigation also enabled a critical assessment to be made of the constructions used in the past to implement the puff-trajectory simulation, for example the techniques of puff merging and puff 'splitting'.

b. The study of deposition in foggy conditions indicated that the enhancement of deposition levels caused by the activation of released particulate matter could have a significant impact on the consequences of accidental releases of radioactivity for some source terms and site characteristics. On the other hand, attachment to existing droplets appears to be an inefficient process. Increased local deposition could be very important for consequence types which involve a threshold, but would also attenuate the amount of material remaining airborne for transport to centres of population downwind. Taking into account both fog frequency and its potential impact, it appears that the emission of foggy conditions could lead to significant distortion of at least some regions of the frequency-consequence curves resulting from a probabilistic study. The explicit inclusion of the effects of fog on consequence calculations would require information on the released particulate relating to its ability to nucleate droplets at fog supersaturations.

c. The work on Lagrangian-particle methods is at an early stage of development.

IV. Objectives for the next reporting period:

The work on the use of Lagrangian-particle techniques to investigate deposition will be continued. After the testing of methods on the homogeneous case, the inhomogeneous atmospheric boundary layer will be tackled, and gravitational settling introduced. Comparisons will be made with eddy-diffusivity theory and with simpler models.

Studies will be commenced aimed at a more realistic representation in consequence-assessment codes of the patterns of wet deposition, especially those arising from heavy rain in conjunction with long-duration releases.

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 a uniform canopy : evaluation of a first-order-closure mathematical model. Atmospheric Environment 21, 1573-1585.

UNDERWOOD B Y (1988). Dry deposition to an urban complex, Radiation Protection Dosimetry (in proof).

Reports :

UNDERWOOD B Y (1987). Dry deposition to an urban complex, SRD R423 Safety and Reliability Directorate, UKAEA

UNDERWOOD B Y (1987). Dry deposition to vegetated surfaces : parametric dependencies. SRD R442. Safety and Reliability Directorate, UKAEA.

UNDERWOOD B Y (1987). On the interpretation of puff-trajectory modelling. Contract report to CEC.

UNDERWOOD B Y (1987). Deposition in foggy conditions. Contract report to CEC.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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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 investigate seasonality of uptake of a discrete deposition episode into green vegetables and milk for releases occurring at different times of the year.

Secondly perform uncertainty analysis on uptake into foodstuffs for scenarios where ingestion dose is dominant using two different techniques.

Thirdly, develop a coupled foodchain/metabolic model for the exposure of the thyroid due to intake of I-131 in milk.

III. Progress achieved:

Foodchain Model Description and Seasonality Studies

The objectives reported here relate to the uptake of I-131 and Cs-137 into fresh green vegetables and fresh, unstored milk. The models employed in the foodchain transfer calculations are simplified versions of the CEGB's general dynamic foodchain model (1). Green vegetable production has been modelled with a 120 day growing season, divided into three 40 day periods and using "average" crop densities for each period to account for the growth in crop density. For milk an outdoor grazing period for dairy cows from early April to October was assumed, with the cows being housed indoors and fed stored feed for the rest of the year.

The seasonality studies were aimed at investigating the dependence of the uptake into green vegetables and milk on the time of year when a discrete deposition of 1E10 Bq/m**2 of I-131 and Cs-137 occurred. In general, the seasonality studies indicate that contamination of green vegetables and milk is most significant for a release in the last quarter of the growing/grazing seasons.

Uncertainty Analysis of Activity Concentration

The source of uncertainty that has been considered in this work is that which arises from the uncertainties in the model input parameters because of the gross variability of the known transfer processes.

The uncertainty analysis was carried out for a discrete deposition of I-131 and Cs-137 occurring just before the harvest of leafy green vegetables and half-way through the grazing period. This scenario maximises the significance of the green vegetable consumption pathway while still retaining the milk consumption pathway as a significant exposure route.

Two methods for uncertainty analysis have been compared. The first, which was preceded by a sensitivity study, was developed by CEGB (2). The second was the Latin Hypercube Sampling (LHS) technique developed by Sandia National Laboratories (3).

The results of the uncertainty analysis have been determined separately for the first year following deposition and for the sum of years 2 to 50 following deposition. Results have also been obtained using different numbers of trials for the LHS technique. The characteristic values of the output probability distribution functions obtained from the two techniques are generally in good agreement.

Coupled Foodchain/Metabolic Model

An expression for the dose-intake factor for the ingestion of I-131 has been derived from first principles using the ICRP model for Iodine. The model has been simplified since the short radioactive half-life of

I-131 means that the organic component has only a negligible impact on the thyroid dose-intake factor. The use of ICRP adult data in this expression yields a dose-intake factor for committed effective dose to the thyroid of 4.5E-7 Sv/Bq, which is consistent with the ICRP30 value of 4.8E-7 Sv/Bq given the assumption that the contribution to thyroid dose from the circulation in the body of the organic iodine component is negligible.

The time-varying dose to the thyroid from milk ingestion has been derived from the expression for the dose-intake factor and the analytical solution for the time-dependent activity concentration derived from the milk model. The resulting analytical expression for the committed effective dose to the thyroid will be used in uncertainty analysis.

(1) S Nair, 1984, "Models for the evaluation of ingestion doses from the consumption of terrestrial foods following an atmospheric radioactive release", CEGB report RD/B/5200/N84.

(2) S Nair and 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.

(3) R L Iman and 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).

IV. Objectives for the next reporting period:

i) Foodchain uncertainty analysis will be extended to additional nuclides and foods.

ii) Coupled foodchain/metabolic models will be developed for more nuclide/food combinations and subjected to uncertainty analysis.

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

It is anticipated that the PRISM method developed by Studsvik will be used as an alternative technique for uncertainty analysis. This will extend an existing collaboration involving a joint analysis of Chernobyl data.

VI. Publications:

Seasonality and Uncertainty aspects of ingestion dose: Some preliminary results:-

Proceedings of a CEC/NEA Seminar on Advances in Accident Consequence Assessment, Rome, 25 - 29 January 1988.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-F-126-F

Institut National de la Santé et
de la Recherche Médicale, INSERM
101 rue de Tolbiac
F-75654 Paris cedex 13

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

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INSERM
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Telephone number: 47.26.5220/5127

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:

. Study the power of the two modified tests of association developed
. Compare their powers to that of a spatial rank correlation test.
. Update the data file.

III. Progress achieved:

In the last two reports we have described modified tests of association for spatially autocorrelated variables X and Y, and simulation models designed to evaluate the performance of the modified tests and compare them to existing procedures. In this report we give results on the power of the modified tests under an alternative hypothesis of linear association between X and Y. The power of a non parametric test of association was also evaluated under the same alternative hypothesis.

Methodology

The modified tests of association were based either on a standardisation W of s_{XY}, the empirical covariance between pairs of observations (X_α, Y_α), $\alpha \in A$, where A is a set of N locations, or on a modification $\hat{H}$ of the degrees of freedom (d.o.f.) of the test on the correlation coefficient r_{XY}. $\hat{H}$ is defined by $\hat{H} = 1 + \hat{\sigma}_r^{-2}$, 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 standardised covariance $W.W = \hat{\sigma}_r^{-1} s_{XY}/s_X s_Y$, is tested as a N (0,1) variable whilst the modified t-test of r_{XY}, $t_{\hat{H}-2}$, rejects the null hypothesis of no association when $|(\hat{H}-2)^{1/2} r(1-r^2)^{-1/2}|$ is larger than the critical value of the t distribution with $\hat{H}-2$ d.o.f.

A non parametric spatial test of association was proposed by Tjøsheim (Biometrika vol 65, 109-114 1978) based on a statistic A which measured the distance between locations of similar ranks for X and Y. The distribution of A given did not take into account the autocorrelation of X and Y.

The power of all three tests was assessed by Monte Carlo simulations. Two independent spatially autocorrelated processes X and W were generated on the grid of the administrative centers of French départements as gaussian variables with a disc model for their autocovariance.

The process Y was defined as $Y = aX + W$. Without loss of generality we could choose $\sigma^2_X = \sigma^2_W$ and hence the correlation ρ_{XY} between X and Y was only dependent on the parameter a. 500 trials were carried out for several levels of autocorrelation in X and W and for the values $\rho_{XY} = 0.2$ and 0.4. The grid contained N = 82 points. Results for higher values of ρ_{XY} are not reported because the power of the tests was very close to 1. The power was evaluated for tests with 5 % nominal level.

Since there is no theoretical reference for the power of these tests, we also calculated the power of the classical test of r_{XY} which would have a similar theoretical variance to the empirical variance of r_{XY}, v_e, estimated by the Monte Carlo simulations. In the case of non autocorrelated variables X and Y, the variance of r_{XY} is equal to $(1 - \rho^2_{XY})^2/N - 1$ for a sample of N observations. For autocorrelated X and Y, we thus computed an equivalent sample size, N^*,

$$N^* = 1 + (1 - \rho^2_{XY})^2/v_e.$$

This number N^* can in turn be used to compute the power β of the classical test of r_{XY} based on N^* observations.

Results

A summary of the results is given in Table 1. On the first line is indicated the empirical variance of r_{XY}. v_e. One can see that v_e is smaller for ρ_{XY} = 0.4 than for ρ_{XY} = 0.2 for the same autocorrelations ρ_X and ρ_W of X and W. This is similar to the case of non autocorrelated variables. Further v_e increases noticeably with increasing ρ_X and ρ_W. N * and β are given respectively on the 2nd and 3rd live. The power of the test W on the covariance is given on the 4th line ; that of the test t_{N-2} is not given because it was nearly identical to that of W. Clearly the tests W or t_{N-2} perform satisfactorily as their power is comparable to β.

The power of the non parametric statistic A was also evaluated. Its values were considerably smaller than those of the modified tests in most cases (from 5 % to 16 %). In the most autocorrelated case (0.8 x 0.8), the observed type I error for the statistic A is around 13 % instead of the 5 % nominal level. Even in this case, the power of the statistic A was only 13 % and 16% for ρ_{XY} = 0.2 and 0.4 respectively, thus not larger than that of the modified tests even though the type I error was larger.

Conclusions

Monte Carlo studies have shown that the power of the modified tests of association developed is good as it is close to that of the standard test based on a sample size which would lead to a comparable empirical variance of the correlation coefficient. Further, for an alternative hypothesis of linear association between X and Y, their power is much greater that that of a spatial rank correlation test developed by Tjøsheim.

Data updating and applications

The data file was updated by inclusion of the percentage of workers in specific industrial branches recorded by census in 1962 for each département. In table 2 are presented results of tests of association for the modified W and t_{N-2} test and for the standard t-test. One can clearly see how the significance levels are reduced when the spatial structure is taken into account. In particular the link between lung cancer and the textile industry becomes non significant.

Table 1 : Results concerning the testing of the correlation between X and Y = aX + W, where both X and W are spatially autocorrelated. X and W independent and of equal variance and a is chosen so that the correlation ρ_{XY} between X and Y tables the value 0.2 or 0.4 .

ρ_X / ρ_W	ρ_{XY} = 0.2			ρ_{XY} = 0.4		
	0	0.4	0.8	0	0.4	0.8
0	0.01227 N* = 76 0.41 0.44	0.01193 N* = 78 0.42 0.42	0.01345 N* = 70 0.38 0.36	0.0093 N* = 77 0.95 0.96	0.00801 N* = 89 0.98 0.97	0.01059 N* = 68 0.93 0.90
0.4	0.01166 N* = 80 0.43 0.43	0.01485 N* = 63 0.36 0.35	0.01924 N* = 49 0.28 0.22	0.00862 N* = 83 0.97 0.97	0.01188 N* = 60 0.90 0.92	0.01539 N* = 47 0.81 0.75
0.8	0.01134 N* = 82 0.44 0.52	0.01742 N* = 54 0.31 0.37	0.05252 N* = 19 0.13 0.13	0.01005 N* = 71 0.94 0.96	0.01689 N* = 43 0.77 0.87	0.03622 N* = 20 0.43 0.44

In each cell are represented from top to bottom : v_e . the empirical variance of r_{XY} (500 simulations) : N* . the estimated d.o.f. based on v_e ($N^*-1=(1-\rho_{XY}^2)^2 / v_e$) : β . the power of the standard test of the correlation coefficient based on N* independant observations : the power of the test based on the standardised covariance W.

Table 2 : Comparison of the significance levels for tests of the association between lung cancer mortality rates and several risk factors given by standard test, W and $t_{\hat{N}-2}$ tests.

* standard test (t transformation with 80 d.f.)

	r	t_{N-2}*	W	$\hat{N}$	$t_{\hat{N}-2}$
Cigarette sales per inhabitant (1953) (CC)	0.76	10.48 $p \leq 10^{-21}$	2.98 p=0.0032	15	4.22 p=0.001
% male workers in metal industry (1962) (MM)	0.63	7.16 $p \leq 10^{-11}$	2.49 p=0.0136	16	3.00 p=0.01
% male workers in textile industry (1962) (TW)	0.28	2.57 p=0.01	1.51 p=0.13	30	1.52 p=0.15

IV. Objectives for the next reporting period:

- Update the data file by inclusion of :
  . cancer mortality rates for a recent period
  . available data on background radiation
- Using the statistical techniques developed, study the joint variation of low dose radiation, industrial pollution and mortality for cancer of specific sites.

The file will contain mortality data at three time points thus allowing the checking of the coherence of the results over time as well as a sufficient time lag between the risk factors and their potential health effects.

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:

No publications.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no.: B16-F-110-UK

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

Ms. M.D. Hill
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Chilton, Didcot
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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

Head(s) of project: M D Hill

Scientific staff: S M Haywood, C A Robinson

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:

In this year's work, further consideration was to be given to problems related to the definition of a critical group in an exposed population. The variation in dose received by individuals in a population, due to variation between individuals in such things as habit and metabolism, was to be investigated. The aim of the work is to identify which factors are likely to most influence critical group doses, for a number of key radionuclides and exposure pathways, and to generalise any conclusions into a more quantitative approach to the identification and definition of a critical group.

III. Progress achieved:

1. Methodology

Available statistical techniques can be used to investigate the distribution of dose in a population, by considering the distributions of factors which affect the dose received, such as habits, dose per unit intake and concentrations of radionuclides in the environment.

This year, attention has been limited to considering the variation between individuals in the consumption of a number of important foodstuffs, and dose per unit intake for several important radionuclides following ingestion. When sufficient information on these are available, an analysis of the combined distribution - representing population dose - will be made using Latin Hypercube Sampling techniques, and the factors most influencing the higher doses will be determined.

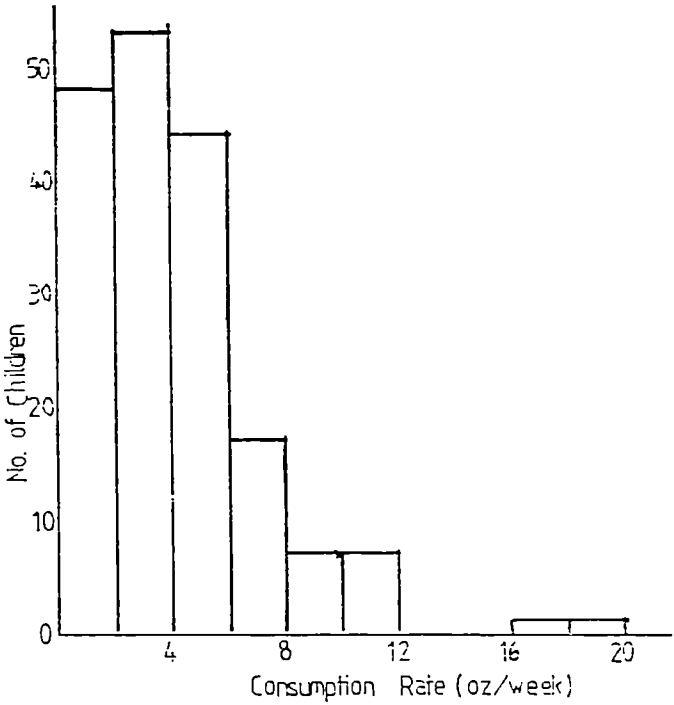
2. Results

Available information on frequency distributions of consumption rates of food in the UK has been reviewed. There are two principal sources of this, results from the Ministry of Agriculture, Fisheries and Food's National Food Survey and an NRPB study of food consumption data for pre-school children, obtained from the UK Department of Health and Social Security and analysed during the previous year's work on this contract. These sources have provided considerable data on the expected distribution of intake rates of key foodstuffs in a population, and the analysis is still continuing. An example of this information is attached.

Less information is currently available on the variation between individuals in the dose they receive following intake. This area is currently being reviewed by staff in the Physical Dosimetry and Biomedical Effects Departments of NRPB, with the aim of obtaining probability distributions for doses per unit intake for several key radionuclides. Consideration will be given to variation with age, and possible correlations with intake rate.

3. Discussion

Much of the necessary input data has been collated this year. The statistical programmes which will be used to analyse the data now exist in a suitable form, and preliminary runs using test data have been done. The full analysis awaits the outcome of the study of the variability in dose per unit intake between individuals, being undertaken at NRPB.



Histogram of mean consumption rates for total meat by infants aged 6-18 months

IV. Objectives for the next reporting period:

The work described above will continue over the next year. It will be broadened to consider the effects of other aspects on population dose, such as variability in the concentration of the radionuclide in food, and an examination of pathways other than ingestion, such as external exposure. The implications of the results obtained for the present methods for identifying critical groups around nuclear sites will be considered.

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: M D Hill

Scientific staff: A D Wrixon, J Croft, A Hudson, D Hart, 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 1987 were: (i) to continue the development of practical guidance for the implementation of each step of the ALARA procedure in those studies which had been defined during the previous years of the contract, and (ii) to consolidate the practical thinking associated with the various case studies carried out in previous years.

III. Progress achieved:

In the past, the work had involved two main aspects: (a) the development of practical guidance and drafting of a report; and (b) the definition of new case studies to be integrated into the main text of the report.

At the beginning of 1987 it was decided that, from the point of view of writing up the report, the work on the case studies could be considered as more than adequate. It was therefore agreed that effort should be devoted to consolidating the practical thinking associated with such studies, focusing on how the ALARA concept can be practically implemented in operational situations, and particularly on the value of using a structured approach and the problem of collecting relevant dosimetric data. The roles of ALARA audits, quantitative decision-aiding techniques and predictive ALARA plans have also been clarified as a result of this exercise. (See 'Key issues in the implementation of ALARA in operations' by J Lochard and J R Croft, submitted for publication to the Journal of the Society for Radiological Protection.)

The brief outline of the report on the practical implementation of ALARA, which was produced during 1986, was extended and modified during 1987. The structure and presentation of the report have been agreed in detail, and references and relevant sources of information have been identified. The case studies will be incorporated within the text, wherever possible, and it is envisaged that there will be a section at the end of the report, where a few examples will be worked out in full in order to clarify the application of the ALARA procedure.

Drafting of the full report has been started, and the intention is to produce the first version during the initial half of 1988. It is expected to run to more than 100 pages. A structure for the report is given in the attached.

STRUCTURE OF THE REPORT

1. Introduction

What is ALARA?

- Historical perspective: An extended abstract discussing the past and the present outlook, introducing the subject
- Concepts underlying ALARA: No threshold; linear dose-response relationship; principle of resource allocation.
- The ALARA procedure: Brief explanation of methodology
- Different levels of decision/application: Design, operational, regulatory authority, political.
- ALARA and practical radiation protection: Fields of application; identification of potential for dose reductions/resource allocation; overview of present situation.

2. The ALARA Procedure

2.1 Structuring the problem

[a] Analysis of the problem

- Definition of system to be studied, ie identification of the nature of the problem and the boundaries.
- Importance of the level of decision.

[b] Analysis of the decisional factors

- Identification of relevant factors and interactions between them.
- Identification of non-quantitative factors to be included in a sensitivity analysis.

[c] Identification of protection options

- Factor by factor approach to ensuring no important options are omitted.
- Interdependencies.

2.2 Quantification of factors for each option

[a] Financial costs of radiological protection

- Costing procedures; discounting; truncation.
- Interactions between protection and other costs.
- Level of detail required.

[b] Radiological detriment to health

- Factors to be quantified.
- Data requirements.
- Methods and models for calculations.
- Long term effects.

[c] Other factors

- Benefits other than radiological protection.
- Non-radiological risks
- Non-health detriment.
- Probabilistic risks.
- Risk aversion.

[d] Data collection

2.3 Comparison of options

- General description and review of decision-aiding techniques. Cost-effectiveness, CBA, decision analysis and multi-attribute utility analysis (MUA). Distinction between aggregative and comparative approaches.
- Choice of technique
- Dealing with judgements

2.4 Sensitivity and uncertainty analysis

- Methods.
- Use of results.

2.5 Presentation and interpretation of results

- Format of presentation for decision makers.

3. Practical Implementation of ALARA

3.1 Use of ALARA procedure

3.2 ALARA and strategy decision

3.3 ALARA design of installations

Annexes

A simple guide to practical implementation of ALARA, using worked examples that will methodically take the reader through the procedure as set out in this report.

IV. Objectives for the next reporting period:

Having established the structure of the report and completed the work on the case studies required, the aim for the year of 1988 is to prepare the draft final report. This work is currently in progress.

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

CEPN
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F-92280 Fontenay-aux-Roses
France

VI. Publications:

Webb, G A M and Croft, J R, Optimisation of public and occupational exposure. The Sizewell B Study. Paper presented at the American Health Physics Society mid-year Symposium. December 1987, Miami.

Croft, J R, L'Optimisation de la radioprotection des patients; elements du réflexion. Accepted for publication by the French journal Radioprotection.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

National Radiological
Protection Board
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, M E Morrey, 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 work forms part of the MARIA-2 Programme (Methods for Assessing the Radiological Impact of Accidents).

II. Objectives for the reporting period:

The choice of dispersion model could have implications for the models to be used in other parts of the consequence calculations, eg use of a trajectory model implies that the dose calculation and countermeasures models may need to consider the plume passing over a single point on more than one occasion. The work during this period centred on a study of the possible applications of accident consequence modelling, and the types of dispersion model which should be used. Particular attention was paid to non-linear trajectories, and how the results of such models compare with those of linear models.

III. Progress achieved:

The MARC program was modified to include a non-linear trajectory model for atmospheric dispersion. This change involved modifying the later parts of the code in addition to the dispersion module. The trajectory model adopted was based on the Riso code RIMPUFF.

RIMPUFF is a Guassian puff model. Each puff has an equal proportion of radionuclides and they are released at regular intervals. The puff centres are separately tracked using meteorological data from one or more stations interpolated to the puffs' respective positions. For use as a linear model, the wind direction is a constant, and data from only one meteorological station is used.

In order to reduce the amount of computation needed, but to derive valid results, the analysis was limited to those effects which only occur within a fairly short distance of the site. Therefore, the end-points considered were the predicted number of early deaths, number of people evacuated and areas heavily contaminated.

Using an interpolated meteorological field requires a large amount of data. Therefore the results using data from ten stations and from just one were examined, as was the distance apart the respective puffs would be using the two different sets of data.

The computing time required by RIMPUFF is approximately proportional to the number of puffs used. The effect on the concentration contours around the site was investigated, and the final effect on the PRA end-points of using different numbers of puffs was examined.

The meteorological sampling scheme in MARC had to be adapted for the trajectory runs. To obtain a full windrose in the sequences used, the bins obtained under the original sampling scheme were further divided into six wind directions around the site.

Using a VAX mainframe computer the multiple puff trajectory model took about ten times longer than the linear model. The differences in the end-points were significant but not large, apart from the high percentiles. This could be because of the greater number of sequences used in the linear model - equivalent to 36 times greater.

Though, at times, great differences were found in the puff trajectories using single station meteorological data or multiple station meteorological data, these did not affect the results of the PRA run which were strikingly similar. This is probably because most early effects occur

fairly close to the site where the site meteorological conditions dominate the interpolated conditions.

A single puff does not model the release adequately. The concentration distributions close to the site were clearly different when the wind direction changed. This was true even when the change was as small as 30 degrees which occurs about 10% of the time. The number of early deaths predicted was about twice that predicted by the other models.

In conclusion, the number of early effects is not greatly affected by the choice of linear or trajectory model, though significant differences were found. The extra computing cost of the trajectory model is at least ten times that of the linear model. Unless this is an unimportant factor, the linear model would be preferred on these grounds.

IV. Objectives for the next reporting period:

The work in the next reporting period will centre 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.

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

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Institut fur Neutronenphysic und Reaktortechnik
Postfach 3640
D-7500 Karlsruhe 1
Bundesrepublik Deutschland

VI. Publications:

None

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, M J Crick, J Brown, A Walmsley

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 feshwater 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 main objective was to specify the structure of the data bases required by a PRA code and the calculations to be performed within the code in order to accurately model urban exposure in the optimum manner. In addition, the urban dose code already developed was to be extended to enable decontamination to be modelled.

Selected data sets of environmental measurements taken following the Chernobyl reactor accident were to be used to try to validate the NRPB's dynamic model for the transfer of radionuclides through terrestrial food-chains, and the implications of these results for PRA were to be assessed.

III. Progress achieved:

1. External exposure resulting from urban contamination

The computer model to predict external doses from activity deposited in the urban environment is called EXPURT (EXPosure from Urban Radionuclide Transfer). Data have been compiled on the uncertainty associated with the various input parameters and a sensitivity analysis has been performed on the model predictions in order to identify those parts of the model requiring further study and those parameters that are most uncertain. The per caput doses and dose-rates in the urban environment as a function of time after an initial deposit of gamma emitting material were evaluated. The results indicate that our subjective estimate of the uncertainty in predicted dose-rates and doses is about a factor of ten. When compared with the simple model currently used at NRPB the subjective estimates of dose predicted by EXPURT are systematically lower by factors between 5 and 50 at all times. However, the simple model curves run parallel to the EXPURT curves, and could be brought into line with the subjective range of urban doses by changing the shielding factor of 0.5 to a more realistic value of 0.1, the value that has more recently been used at NRPB. At present, at least from this rather limited study, it appears that there is no definite benefit to be gained in using EXPURT routinely, compared with the use of the old methodology assuming a shielding factor of 0.1.

The most important parameter uncertainties were identified as the fraction of ground that is paved, the fraction of time spent indoors by the majority of the population and the ratio of dry deposition on paved areas to that on grass.

EXPURT has been extended to enable it to represent several techniques for decontaminating the various surfaces in the urban environment. These include hosing of impermeable surfaces, such as walls, roofs and paved areas, the removal or ploughing of soil/grass areas and the replacement of building surfaces with new uncontaminated materials. The model has been used to examine the effectiveness of these various decontamination measures in reducing the external radiation doses to people living in urban areas. The importance of the time at which decontamination is performed has also been studied.

2. The transfer of radionuclides through terrestrial foodchains

The predictions of the NRPB foodchain models FARMLAND have been compared with measurement data following the Chernobyl accident, both averaged over large areas of the UK and also for two specific farms in the

UK. Comparison with appropriate sets of data will assess the ability of FARMLAND to represent the general conditions for which it was developed, and also test its ability to simulate site-specific conditions.

For comparisons of model results with environmental monitoring data made after the Chernobyl accident the radionuclides iodine-131, caesium-137 and caesium-134 have been considered for the foods milk, green vegetables and lamb. For the two dairy farms, one in Cumbria and the other in Berkshire, detailed measurement data of deposition and activity concentrations in animal fodder and milk are available for the above radionuclides.

Comparisons of FARMLAND model results with measurement data applicable for large regions of the UK have shown that the models predict the time dependence well. In using the post-Chernobyl monitoring data there are considerable uncertainties in the compatibility of the deposition measurements with those in food. It is often not possible to draw conclusions on the ability of the model to predict the scale of transfer to a particular food from these data, because many of the differences can be attributed to these uncertainties. From this point of view these data are disappointing for detailed model validation.

Comparisons using site-specific data, where uncertainties in interpretation are reduced, have shown that there may be factors influencing the transfer to milk which might be relevant in applying the model to more general conditions; one of these is the equilibrium transfer factor to milk for iodine. Results suggest the possibility that caesium deposited in dry conditions may be less available for transfer than that deposited during rain, at least in the first few weeks.

IV. Objectives for the next reporting period:

The work in the next reporting period will centre on the need to decide what models should be included in the MARIA code, and to plan the specification and outline of the code.

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

Kernforschungszentrum Karlsruhe GmbH
Institut fur Neutronenphysic und Reaktortechnik
Postfach 3640
D-7500 Karlsruhe 1
Bundesrepublik Deutschland

VI. Publications:

Crick, M J, Brown, J, Hussain, Z and Walmsley, A, Identification of important parameters in urban dose assessment. Radiation Protection Dosimetry (to be published).

Brown, J, Haywood, S M and Wilkins, B T, Validation of the FARMLAND models for radionuclide transfer through terrestrial foodchains. Presented at CEC Workshop on methods for assessing the reliability of environmental transfer model predictions, Athens 5-9 October 1987.

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 Haywood, J A Jones, J Brown

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:

This was to involve the application of existing models to examine the effectiveness of particular countermeasures, and the optimum timing and duration of emergency actions. Work on the calculation of dose saved by bringing animals indoors was to continue, and similar work undertaken on the optimum duration of relocation. Work was also to be carried out on other aspects of the costs of accidents, such as decontamination. The option of using derived levels rather than doses as the criteria assumed to be used for introducing countermeasures was to be considered.

III. Progress achieved:

The urban deposition and decontamination work is described in the progress report for Project 2.

Work has continued on the improvement of models for calculating the economic consequences of accidents. A critique of the current economic models used by NRPB and the US code MACCS was prepared by Dr C Heady of University College, London and has been reviewed at NRPB. The critique also gives advice on which aspects of each model should be retained in the improved model. Work has started on the development of a model for costing decontamination. A procedure for incorporating this into an accident consequence code has been developed and data on the costs of decontamination techniques are being reviewed. Data on the costing of health effects are also being reviewed, as is the available information on land-use data in the UK. In particular, the possible use of satellite information and aerial photography in the updating and improvement of the present UK land-use grids is being considered.

A version of MARC has been developed in which the criteria for banning food is the introduction of restrictions above a certain contamination level, rather than in relation to annual dose.

IV. Objectives for the next reporting period:

The work in the next reporting period will centre on the need to decide what models should be included in the MARIA code, and to plan the specification and outline of the code.

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

Kernforschungszentrum Karlsruhe GmbH
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D-7500 Karlsruhe 1
Bundesrepublik Deutschland

VI. Publications:

None

Title of the project no.: 4

Uncertainty analysis

Head(s) of project: M D Hill

Scientific staff: M J Crick, J A Jones, M E Morrey, J R Simmonds

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:

A demonstration uncertainty/sensitivity analysis was to be performed on the new urban dose model using the Sandia LHS program in order to assess the advantages and disadvantages of the technique in analysing environmental transfer models.

Further work of both a computational and theoretical nature was to be pursued on sub-models of MARC, namely the atmospheric dispersion module, the meteorological sampling routines and the countermeasures models. Trial runs were to be carried out for an uncertainty analysis on the whole of the MARC code to investigate its feasibility and to identify any problems.

III. Progress achieved:

An uncertainty analysis on the atmospheric dispersion module of MARC has been carried out. Eariler work carred out under MARIA-1 examined the uncertainty in predicted concentration and deposition at different grid points. This work extended the earlier work by investigating the uncertainty in the final end-points (numbers of health effects, numbers of people evacuated, and amounts of food banned). The subjective range of uncertainty was calculated using the statistical tolerance method adopted in earlier MARIA work. The ratio of upper and lower limits of the uncertainty bands at different percentiles of the probability distribution were found to be generally small (factors of about 8). Analysis in terms of correlation coefficients and regression coefficients between the output and input parameter values was carried out in an attempt to identify those variables which make a major contribution to the uncertainty. Unfortunately this analysis did not give clear results, suggesting that a number of parameters might give similar contributions. The ranges of uncertainty found for the amounts of food banned are comparable to those found in earlier studies in MARIA-1 when only the uncertainty on food-chain parameters was considered.

The work on uncertainty in dose in urban areas is described in the progress report for Project 2.

IV. Objectives for the next reporting period:

The main part of the work will centre on the planning and design of the MARIA code. This code needs to be designed in such a way that uncertainty analyses can easily be performed. An analysis of the uncertainty in the whole of MARC, at least for a release of a few nuclides, will be carried out, as an input to the MARIA code design.

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

Kernforschungszentrum Karlsruhe GmbH
Institut fur Neutronenphysic und Reaktortechnik
Postfach 3640
D-7500 Karlsruhe 1
Bundesrepublik Deutschland

VI. Publications:

Crick M J, Hofer, E, Jones, J A and Haywood, S M, Uncertainty analysis of the food-chain and atmospheric dispersion modules of MARC. NRPB Report. In press.

Crick, M J, Hill, M D and Charles, D, The role of sensitivity analysis in assessing uncertainty. IN Proc. NEA Workshop on Uncertainty Analysis for Performance Assessments of Radioactive Waste Disposal Systems. Seattle, February 1987. OECD Paris (1987).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: B16-F-111-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)]:

Prof. Dr. W. Jacobi
Institut für Strahlenschutz
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Ingolstädter Landstrasse 1
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Dr. G. Drexler/Dr. H.G. Paretzke
Institut für Strahlenschutz
GSF
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 exposure-time-risk surfaces for the GI- tract, pancreas, liver and breast tumors
- comparative analysis of the induction of lung cancer in rats by neutron-, gamma, and alpha-radiation with respect to their relative biological effectiveness

b) analysis of quantitative optimization strategies used in administration and legislation.

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.

- The epidemiological study on the German patients treated with Ra-224 was continued in collaboration with the Kinderpoliklinik der Universität München.

- 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.

b) Numerous papers and books published in the open literature on cost-benefit analyses in the public domain where evaluated.

Results:

a) - Cancer risks were calculated for the GI-tract, the pancreas, the liver, and the female breast as functions of dose, age at exposure, time since exposure, and gender. The estimations were based on linear dose response relationships; for the extrapolation of observed risks to life-time risks the relative risk model was used, thus assuming a proportionality between radiation induced and base-line risks. Examples of the results are shown in figure 1.

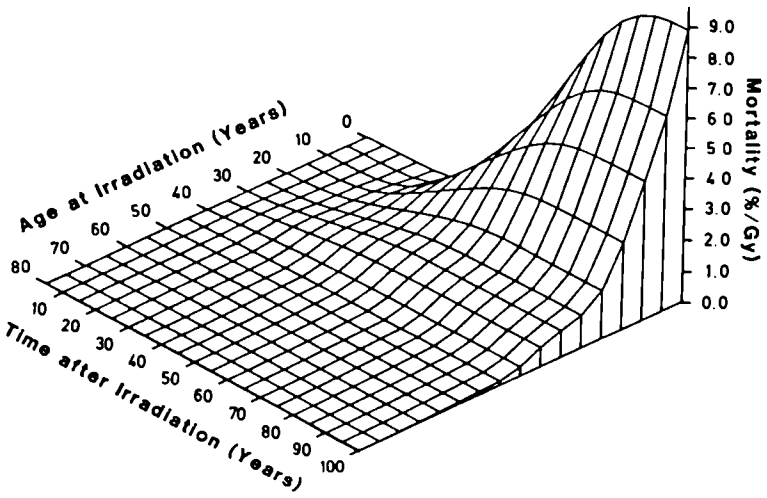


Fig.1: Cumulative mortality from radiation induced breast cancer (females)

- The present analysis of the radon inhalation study at Razes and in Fontenay-aux-Roses (CEA) includes only recent experiments for which a new radon dosimetry was used. Experiments in the intermediate and high dose range are still not completed. The present results based only on low dose experiments give an equivalence ratio of 1.5 WLM of radon to 1 mGy of neutrons compared to the earlier value of 3 WLM/mGy.
- A recent analysis of bone sarcomas in Ra-224 patients in terms of a log-normal distribution of times to tumor appearance has essentially confirmed the independence of this distribution on dose and on age at injection. Another essential result is that the dose response relationship is best described by a linear-quadratic function.

b) In decisions based on previous cost-benefit considerations in the administrative area usually not only internal costs and benefits are taken into account but also external quantities (e.g. use of streets for the construction of a dam) as well as spin-offs (as e.g. higher values of housing lots after improvements on the public transport system in a region). Environmental impact costs, however, are often neglected. "Intangible (e.g. social) costs are qualitatively evaluated but no conversion into monetary quantities is performed.

Discussion:

a) - The results obtained stress the importance of the choice of the temporal projection model for derivation of lifetime-risks; the relative risk model gives risk estimates which are about 3-4 times higher than those calculated on the basis of the plateau-model. Another problem of equal importance is the question how observed risks, relative or absolute, are to be transported between populations of differing base-line risks, and the existence of a dose rate reduction factor for radiation carcinogenesis in man.
- The analysis of animal experiments should be continued by a comparison of the neutron and gamma irradiations in their effectiveness to induce other neoplasms such as vascular sarcomas or carcinomas of the urinary tract. A central point will be the analysis for possible characteristic differences between appearance times of carcinomas and sarcomas.
- The analysis of bone sarcomas has shown that the log-normal distribution of times to the tumor is an adequate model. The dose response relationship found here indicates a reduction by a factor of two in comparison to earlier risk estimates for Ra-224 used in the Radioepidemiological Tables published by the US-NIH.

b) The problem of expressing social or health effects and monetary values in comparable quantities which is encountered in quantitative cost-benefit-analysis in radiation protection is found also in decision making processes in the administrative area; however, there these different quantities are not converted into the same quantities (e.g. monetary units) but compared in a multi-criteria-decision approach.

IV. Objectives for the next 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 seduction strategies employed in limitation of risks from chemical pollutants.

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 cataracts in patients injected with Radium-224.
Accepted for publication in Rad. Res.

D. Chmelevsky, A.M. Kellerer, C.E. Land, C.W. Mays, and H. Spiess:
Time and dose dependency of bone-sarcomas in patients injected with Ra-224.
Accepted for publication in Rad. Env. Biophys.

D. Chmelevsky, J. Lafuma, J. Chameaud, M. Morin, A.M. Kellerer:
Pulmonary Carcinomas in Sprague-Dawley rats after exposure to low doses of radon-daughters, fission-neutrons or gamma-rays.
Submitted for publication to Rad. Res.

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:

Prof. Dr. W. Jacobi and H.G. Paretzke
GSF - Institut für Strahlenschutz
Ingolstädter Landstraße 1, D- 8042 Neuherberg
AG Risikoanalyse (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 types of homes and in open air by means of Monte-Carlo methods for various relevant gamma emitters in the soil and on walls of constructions,
- 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 from Sr, Tc, Cs, I, U, Pu, Am, Np, Cm due to consumption of contaminated foodstuffs by members of the public,
- Assessment of the reliability and variability of these dose-factors.

II. Objectives for the reporting period:

a)- Calculation of shielding factors for different models of building structures and comparison with results from point kernel methods. Experimental validation of calculated shielding factors. Tabulation of nontrivial time dependencies of contributions of daughter nuclides to the external exposure from radionuclides on the ground.

b) Calculation of further dose factors, integration of new values of absorbed fractions recently published by ORNL into these dose calculations; assessment of the reliability and the variability of the results.

III. Progress achieved:

1. Methodology

a) The kerma in certain homes and in open air for gamma emitters in air or deposited on the environment and on the house was determined by detailed Monte-Carlo photon transport calculations employing the SAM-CE code.

b) Dose conversion factors were calculated on the basis of specific absorbed fractions contributed by ORNL. Numerous publications were reviewed to derive data describing the biokinetics of those radionuclides in the human body.

2. Results

a) Using the results of the Monte-Carlo simulation of the photon transport in the urban environment, gamma ray exposures were calculated for various locations inside and outside house for different deposition patterns (dry/wet) and different times after deposition. It was found that due to the relatively high deposition of radionuclides on trees the external exposure in outside locations may be higher by a factor of two than over lawns. For dry and wet depositions with the same contamination of a lawn, the external exposures in most of the urban locations is higher by a factor of 2 - 3 for the dry deposition than for the wet deposition. In urban environments without vegetation the radiation exposures are relatively low. In such cases the exposure indoors may be strongly influenced by radionuclides which were deposited inside the house.
Non-trivial time-dependencies of contributions of daughter nuclides to the external exposure from radionuclides on the ground have been tabulated.

b) Dose conversion factors are now available for most types of radionuclides which could become relevant for the assessment of reactor accident consequences. They were calculated for six age groups as functions of time since incorporation. An example of the results is shown in figure 1.

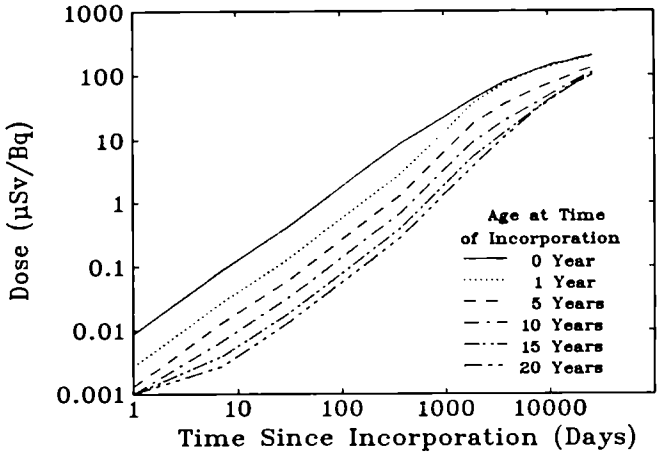


Fig. 1: Dose to the bone marrow after inhalation of 1 Bq of Pu-239 (inhalation class Y)

3. Discussion

a) For many locations the dose rate was found to have a similar dependence on time after deposition as the dose rate over a lawn. Therefore the dose rate over a lawn can be used as a reference in the definition of factors, that describe the influence of the environment on the external exposure. Since the relatively high deposition on trees may lead to higher gamma dose rates in urban environments than over lawns, the terminus 'shielding factor' may be not suitable in certain scenarios. It is suggested to define an 'environment factor' by the ratio of the external exposure rate at a given location to the exposure rate over a lawn at the same time after the deposition.

   The study showed that a better quantitative understanding of the deposition on trees and inside houses is necessary for further improvements on the accuracy of estimates of external exposures from environmental contaminations with gamma emitters. Also more detailed data on the time-dependent decrease of contaminations of the various urban surfaces after wet and dry deposition of radioactivity are needed for more reliable predictions of the external doses and their variabilities.

b) The uncertainty of dose conversion factors resulting from uncertainties of the specific absorbed fractions is less than 50 %.

   The uncertainty resulting from the lack or the unreliability of biokinetic data is expected to be far more important, but it cannot yet be quantified for most of the radionuclides.

IV. Objectives for the next 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 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.

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

NRPB-Chilton (Drs. Dennis, Stather, Adams), CEA-Fontenay-aux-Roses (Drs. Nenotand, Parmentier), Riso-Laboratory (Dr. Hedemann Jensen), Universität Toulouse (Prof. Blanc), ORNL-Oak Ridge (Drs. Eckermann, Christy).

VI. Publications:

P. Jacob and H.G. Paretzke:
Dose-rate conversion factors for external gamma exposure.
Nucl. Instrum. Meth. A 255 (1987), 156 - 159

R. Meckbach, P. Jacob and H.G. Paretzke:
Shielding of gamma radiation by typical European houses.
Nucl. Instr. Meth. A 255 (1987), 160 - 164

Title of the project no.: B16-111-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:

- Laboratory and small scale operational field tests of a prototype partial body dosemeter according to present standard requirements.
- Continuation of development of a film/TLD badge and a work place specific neutron/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. Tentative correlation with biological dosimetry.
- Continuation of the evaluation of exposure conditions and dose distributions in angiography and expansion to nuclear medicine.

III. Progress achieved:

Partial Body Dosemeter: The automated TLD system was developed to a prototype level and became subject of a German und US patent; it was handed over to a German firm for preparation of an industrially manufactured equipment. As for the detector, possibilities were studied to reduce optical fading without affecting other dosemeter properties. Further RD activities referred to a system application in beta radiation dosimetry, particularly H'(0.07) assessment, without allowing for changes in handling, procedures and readout equipment. The new system was involved in the annual quality control in personnel monitoring through the PTB, Braunschweig, to demonstrate accuracy and reliability under objective conditions.

Film/TLD badge: Efforts were continued, together with industry, to design a combined personal dosemeter badge provided to integrate a TLD assembly for dosimetry and a film detector for analysis of the radiation field and exposition conditions.

Statistical evaluation of occupational exposure: The statistical evaluation of data from the GSF Personnel Monitoring Service was continued; the findings for the year 1986 were published in the GSF Report 17/87. A compilation of the dosimetric data obtained since 1980 shows, that the collective dose resulting from medical applications remained almost constant at around 8 man-Sv. The collective dose resulting from industrial applications, however, varies remarkably in the course of the years. Nevertheless, an overall reduction of the total collective dose is obvious. The mean annual doses of all persons monitored seem to confirm this development, but the decrease is mainly due to the steady increase in the number of persons monitored. Considering only the "exposed" persons, the situation looks different; accordingly, the mean annual dose of these persons first increased during 1980-1982, decreased then and finally remained constant since 1984.

Assessment of lifetime doses: In order to assess individual dose histories and lifetime doses, the personal data and the dosimetric findings for a subgroup of monitored persons were put in the computer for the years 1960 through 1980. This subgroup of about 17 000 individuals consists of the persons who were monitored with the "universal-badge". 130 of such persons mainly working in research establishments and nuclear power plants were identified to be registered for 15 years and more. In this group "dose prones", i. e. persons who persistently receive high annual doses for periods of 5 to 10 years, could be identified; table 1 shows some examples. According to a proposal of Johnston et al. (1), a statistical indicator D_k was used to quantify the difference between an individual's annual doses and those of the group as a whole. D_k is a measure of the distance of each workers's dose history from the mean of the group. A value of $D_k > 1$ means that during the time period taken into account an individual on average receives doses of greater than one standard deviation above the respective mean annual dose of the whole group.

Table 1: "Dose Prone Individuals": Time interval: 1969 - 1973;
number of persons: 130;
average of mean annual doses: 8.44 mSv

Name	Mean Annual Dose (mSv)	D_k
B. A.	48.04	2.714
B. K.	48.12	3.208
C. P.	41.20	2.334
J. E.	48.10	2.734
R. A.	40.76	2.305
R. W.	39.48	2.290
S. A.	40.88	2.120
S. R.	36.06	2.297
W. O.	45.00	2.507

(1) P. D. Johnston, J. Brenot and G. M. Kendall: "A Study of Worker Dose Distributions with Respect to ICRP Dose Limitations", Health Phys. Vol. 51, No. 5, pp 579-599 (1986)

Workplace analysis: In order to determine workplace specific exposure models for occupationally exposed persons, the measurements at selected workplaces in angiography and, to a minor extent, in nuclear medicine were continued. The results obtained in angiography showed that the effective dose equivalent is underestimated if the measurement is made below the lead apron. A side effect obtained was a remarkable reduction of the hand exposures especially in cases, where extremely high values were found in 1986; as an educational effect of our continued dose reports, the doses, for instance, decreased from monthly values up to 90 mSv to values around 5 mSv and below.

In nuclear medicine, the individual doses measured at the trunk were shown to give an accurate estimate of the whole body exposure. However, values of hand exposures were found up to thirty times higher than the whole body doses.

IV. Objectives for the next 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.

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

VI. Publications:

Drexler, G., Eckerl, H., Haid, G., Scheibe, D.: Statistische Ergebnisse aus der amtlichen Personendosisüberwachung 1985. Auswertungsstelle für Strahlendosimeter. GSF-Bericht 17/87 (1987)

Brand, H.N.: Fingerring Dosimeter. Deutsches Patentamt: Gebrauchsmuster Nr. G 8510060.9; United States Patent Nr. 4.698,505

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor.

Technical University of Denmark
Laboratory of Applied Physics I
DK-2800 Lyngby

Contract no.: BI6-F-113-DK

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 on the following:

A) Space charge modification of electric fields.
A theory is developed for the modification of electric fields by the presence of space charges.

B) Plateout studies.
The plateout of individual radon daughters on metal discs exposed to "deformed" positive electric fields has been studied together with the effect of the plateout on the remaining airborne, attached and unattached radon daughters.

III Progress achieved.

A) Space charge modification of electric fields.
The theory for the variation of the electric field around an ionizing electrode is developed, and it is demonstrated that the variation with distance is largely determined by the space charge created by the ions. In the (theoretical) case of a spherical system it is demonstrated that with a non-ionizing electrode the field strength in 96 % of the volume is less than 10 % of the mean value, while in the case of an ionizing electrode the field strength in no point of the room is less than 50 % of the mean value.

The essential features of the theory are substantiated by measurements in an experimental room. With a single (spherical) electrode suspended from the ceiling it is demonstrated that the field strength at the boundary of the room is about 4 times higher, when the electrode is ionizing than when it is not.

B) Plateout studies.
1. Methodology.
The (plateout) effect of electric fields on airborne radon daughters was studied in the following way. In a 150 m^3 room with a radon concentration of about 1000-1500 Bq/m^3 a system of four corona emitters was mounted below the ceiling. In order to increase locally the plateout rate over a small area, grounded metal collector discs were placed about 0.40 m above the virtually grounded table top directly beneath the emitters. The fact that the discs are raised above the table will increase (deform) the field at the face of the discs and thus increase the plateout rate on the discs, still keeping the field strength proportional to the voltage of the field-producing electrode.

The following parameters were measured: radon concentration, individual radon daughter concentrations and unattached fractions, aerosol concentration and AMD, corona current from the emitters and plateout rate (number of atoms per unit time) for individual daughters on the discs as a function of the emitter voltage.

2. Results.
The efficiency of the plateout process can for a given daughter product be characterized by the absolute plateout efficiency, p_a, defined as the fraction of the activity produced, which is plating out, and the relative plateout efficiency, p_r, defined as the ratio between the plateout rate and the activity remaining airborne.

The results show that only ^{218}Po and ^{214}Pb are plating out to any significant degree. Further that the plateout efficiencies of ^{214}Pb are about 10 times higher than those of ^{218}Po. Both the absolute and the relative plateout efficiencies will generally increase with the emitter voltage, but in the case of the relative efficiencies the increase is so pronounced around the voltages where the emitters go into corona, that it is beleived to be related to an extra charging of the radon daughters by the corona current.

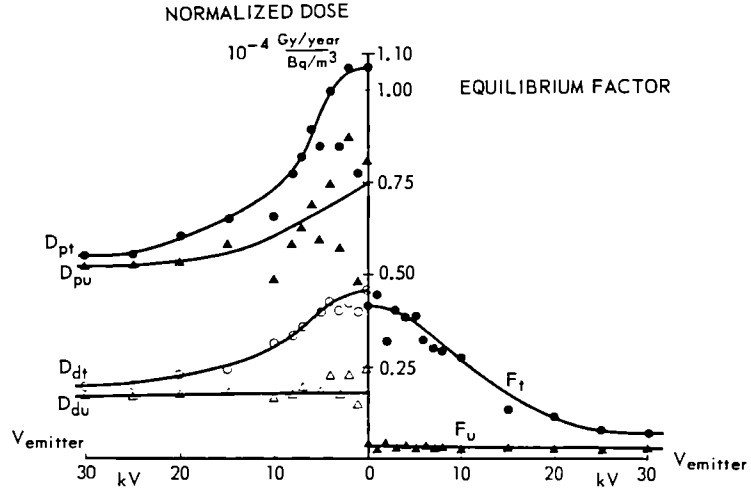
The direct effect of the plateout on the level of airborne radioactivity is shown in the Figure. In the right half is plotted as a function of the emitter voltage the equilibrium factor, F_t, corresponding to the

total daughter population, as well as the equilibrium factor, F_u, corresponding to the unattached daughters.
In the left half are plotted the normalized mean bronchial doses corresponding to the total daughter population as well as that corresponding to the unattached daughters, calculated on basis of individual daughter activities, D_{dt} and D_{du}, as well as those calculated on basis of the total and unattached potential alpha energy concentrations, D_{pt} and D_{pu}.

3. Discussion.
It appears that while the equilibrium factor, F_u, corresponding to the unattached daughters is almost constant about 0.03 independent of the emitter voltage, the total equilibrium factor, F_t decreases from about 0.42 to approximately 0.07 at 30 kV.
These results are also reflected in the variation of the doses with the emitter voltage, with the dose from the unattached daughters being almost constant, while the total dose at 30 kV has decreased to about half of its value at an emitter voltage of 0. At 30 kV 87 % of the total dose is due to unattached daughters.

It appears that only the activity of the attached daughters is affected to any significant degree by the electric field. This may not necessarily mean that unattached daughters are not charged and plating out, but rather that the plateout process itself, by removing aerosol particles from the air, causes more daughter products to stay unattached and thus compensates for the unattached plateout.

By the experiments described the total potential alpha energy concentration was lowered by about 83 % and the radiological dose by about 50 % of their orginal values.



Equilibrium factor and normalized mean bronchial dose as a function of the emitter voltage.

IV. Objectives for the next reporting period:

In the next reporting period the plateout conditions with negative ionization will be studied.
Further the effect of various commercially available ionizers will be investigated under laboratory and more practical conditions.
A small positive ionizer for approximately 20 kV is presently under construction and will be tested alone and in combination with filters of various capacity.

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

dr. J.P.McLaughlin, Physics Department, University College, Dublin, Ireland

VI. Publications:

Niels Jonassen and Bent Jensen: The effect of Filtration on Radon Daughter Atmospheres, Laboratory and Field Experiments,
Second International Specialty Conference on Indoor Radon, APCA,
Cherry Hill, New Jersey, April 1987.

Niels Jonassen: Ions, Space Charge and Fields,
9th annual EOS/ESD symposium, Orlando, Fa, September-October 1987

Niels Jonassen and Bent Jensen: Modification of Electric Fields by Space Charges, Effects on Airborne Radon Daughters,
Fourth International Symposium on the Natural Radiation Environment,
Lissabon, December 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Kernforschungszentrum Karlsruhe
GmbH, KfK
Postfach 3640
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Contract no : BI6-F-128-D

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

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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 objectives 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:

To quantify the implications of using trajectory models in ACA codes, probabilistic comparitive calculations have been carried out with different atmospheric dispersion models. The calculations were part of a benchmark study which also comprised a deterministic comparitive analysis of different types of atmospheric dispersion models. In the probabilistic investigation the influence of different dispersion codes on frequency distributions of activity concentrations, radiation doses and health effects has been demonstrated with the UFOMOD code. Based on the results a new concept of atmospheric dispersion modelling in the new program system UFOMOD has been developed.

III. Progress achieved:

In the last years various atmospheric dispersion models of different complexity have been developed taking into account changes of wind direction. Compared with the straight-line Gaussian plume model conventionally used in ACA codes those models describe the atmospheric conditions and the distribution of concentration more realistically. Since the accuracy of the concentration distribution obtained determines the accuracy of the results of all subsequent submodels of an ACA model, probabilistic comparative calculations with improved dispersion models have been carried out

1. to identify those models which can be applied in ACA codes under the demands of reasonable computer time and availability of meteorological input data;
2. to quantify the implications of different concepts of dispersion modelling on the results of an ACA.

The most important results of these investigations can be summarized as follows:

- there are trajectory models available which can be applied in ACAs, since the increase in computing time is negligible;
- trajectory models provide much more realistic results of ACAs than straight-line Gaussian models;
- trajectory models increase the applicability of ACA codes (e.g. optimization of protective measures).

The conclusion from the comparitive study was to apply trajectory models in ACAs. This led to a completely novel concept of atmospheric dispersion modelling in the new program system UFOMOD. (Fig. 1).

UFOMOD	
Near range model ($\lesssim$ 50 km)	Far range model ($\gtrsim$ 50 km)
Atmospheric dispersion	
MUSEMET (KFA) RIMPUFF (RISϕ)	MESOS (ICST) Windfields in the regions 10°W - 50°E and 36°N - 62°N
10 measuring stations synoptic data of 1982 and 1983 recorded at 1 h intervals	~800 measuring stations synoptic data of 1982 and 1983, recorded at 3 h intervals

Fig 1 UFOMOD. Modelling of atmospheric dispersion

Due to the fact, that

- site-specific characteristics are only relevant in the near range and vanish at farer distances,
- the quality and quantity of consequences in the near range (fast protective measures, early health effects) are different from the far range (long-term countermeasures, stochastic health effects),
- the near range can be modelled much more in detail than the far range, and
- many applications of ACAs refer to only one of both distance ranges,

different ranges of validity are distinguished and assigned to respective trajectory models:

1. the near range (< 50 km), where modified versions of the trajectory models MUSEMET and RIMPUFF, respectively, are used to calculate the spread of concentrations;
2. the far range (> 50 km), where the computer code MESOS is applied, which calculates the dispersion of the radioactive material along precalculated wind fields.

IV. Objectives for the next reporting period:

A further improvement is planned by the implementation of a special simplified atmospheric dispersion model to estimate the spatial concentration distribution for low-level long-duration (weeks or months) releases of radioactive material. To that purpose, the computer code ISOLA will be modified for use in UFOMOD.

To increase the applicability of UFOMOD the behaviour of tritium (HT,HTO) during dispersion and deposition will be modelled in the available atmospheric dispersion codes.

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

National Radiological Protection Board Chilton, Didcot
GB-Oxon OX11 ORQ

VI. Publications:

J. EHRHARDT, K.BURKART, I. HASEMANN, C.MATZERATH, H.-J. PANITZ, C.STEINHAUER
The program system UFOMOD to assess the consequences of nuclear accidents
Karlsruhe, internal report, December 1987, to be published as report KfK-4330

J. EHRHARDT, I. HASEMANN
Ergebnisse der "Deutschen Risikostudie Kernkraftwerke"-Phase B: Modellierung im Nahbereich und Abschatzung nichtstochastischer Schäden
Jahrestagung Kerntechnik, Karlsruhe, 2.-4. Juni, 1987
Tagungsbericht des Deutschen Atomforums e.V., Bonn, P.271-274

H.-J. PANITZ
Accident Consequence Assessments with Different Atmospheric Dispersion Models
Paper presented at the International SNS/ENS/ANS, Topical Meeting on Probabilistic Safety Assessment and Risk Management, Aug. 30-Sept. 4, 1987, Zurich, Conference Transactions, Vol. III, 933-938, Verlag TÜV Rheinland, Köln, 1987

H. J. PANITZ
Probabilistische Unfallfolgenabschatzungen mit unterschiedlichen Ausbreitungsmodellen
Jahrestagung Kerntechnik, Karlsruhe, 2 -4 Juni 1987, Tagungsbericht des Deutschen Atomforums e V , Bonn, P 267-270

Title of the project no.: 2.

The assessment of exposure due to deposited material

Head(s) of project:

Claudia Steinhauer

Scientific staff:

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 MARIA2.

II. Objectives for the reporting period:

The prime KfK objective was to complete those parts of the program system UFOMOD which are concerned with the exposure due to deposited radioactive material, including the assessment of the health effects resulting from this exposure. Another line of effort was the development of supplementary evaluation programs to present the intermediate and final results in a form which allows an easy and efficient interpretation.

III. Progress achieved:

In the reporting period, the development of the program system UFOMOD was completed with respect to the evaluation of doses and health effects. UFOMOD consists now of three independent subsystems (Fig. 1):

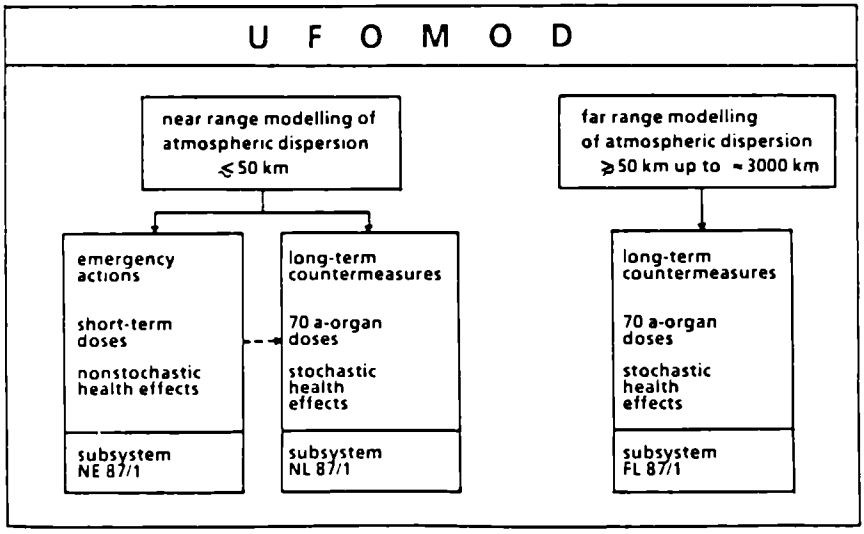

Fig. 1: UFOMOD: General structure of the program system

Subsystem NE is for the assessment of the consequences of acute exposure in the near range (< 50 km), the subsystems NL and FL for the assessment of the consequences of long-term exposure in the near range and in the far range, respectively. The assessments can be made with or without taking possible countermeasures into account.

Output of each subsystem are - among others - individual organ doses and health effects risks, collective doses and the projected number of health effects. Special emphasis was laid on the development of evaluation programs which allow a multitude of graphical and tabular repesentations of the results, for instance distributions of statistical quantities (e.g. expectation values, percentiles) as functions of distance, pie diagrams of the contributions of radionuclides, exposure pathways and foodstuffs. Several two-dimensional diagrams of the projected number of cancer incidences or collective dose against individual risk or dose may be helpful to demonstrate that the major part of the stochastic effects is usually accumulated at low levels of dose and personal risk.

To estimate the number of cancer incidences as a function of time, in UFOMOD the concept of activity - risk coefficients has been introduced. These are pathway dependent coefficients normalized to unit activity concentrations in air or on ground surfaces, and quantify the risk as a function of time of an individual which is representative for the general population.

Mathematically, the activity - risk coefficients are expressed by manifold integrals over age and life expectancy distributions of the population, the age and time dependence of the intake of activity for internal exposure pathways, the age and time dependence of irradiation for all exposure pathways, and of the individual risk. The coefficients are precalculated, reducing the assessment of cancer incidences in UFOMOD to simple multiplications of the coefficients with the initial activity concentrations and with the numbers of individuals affected.

An example for the time-dependency of the number of cancer incidences is given in Fig. 2, where the 95% fractiles of the corresponding distributions are shown as a function of time for two cancer types. Due to different latency and manifestation periods, the highest mortality rates are estimated to occur in time intervals delayed by different numbers of years or decades.

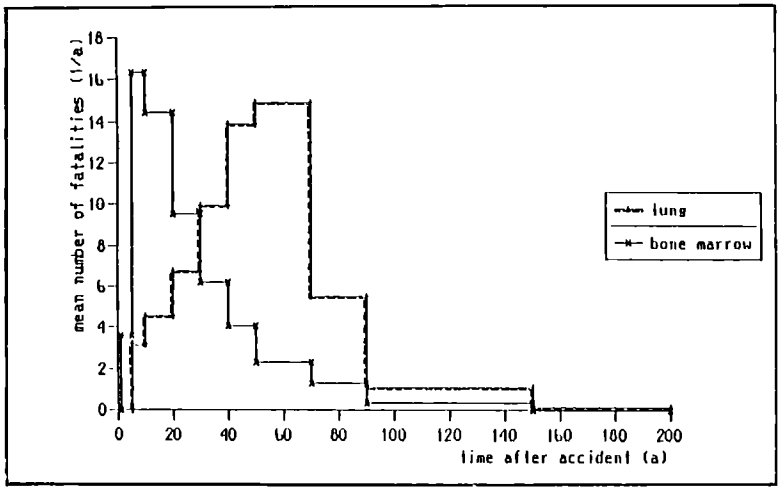

Fig. 2 Examples of the time dependent mean number of late fatalities for different cancer types

IV. Objectives for the next reporting period:

With the new version of UFOMOD accident consequence assessments using new source terms of the GERMAN RISK STUDY - PHASE B will be performed and documented. Work with be started to complete the methodology by the inclusion of ß-irradiation of the skin and models for tritium behaviour in the foodchains

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

National Radiological Protection Board (NRPB)
Chilton, Didcot
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Gesellschaft fur Strahlen- und Umweltforschung (GSF) mbH
Institut fur Strahlenschutz
Munchen Neuherberg
Ingoldstadter Landstr. 1
D-8042 Oberschleissheim

VI. Publications:

C STEINHAUER, C MATZERATH
Ergebnisse der 'Deutschen Risikostudie Kernkraftwerke' - Phase B:
Modellierung im Fernbereich und Abschätzung von stochastischen somatischen Schäden
Jahrestagung Kerntechnik '87, Karlsruhe, June 2-4, 1987,
Tagungsbericht des Deutschen Atomforums e.V., Bonn, p. 275 - 278

J. EHRHARDT, K.BURKART, I. HASEMANN, C.MATZERATH, H.-J. PANITZ, C.STEINHAUER
The program system UFOMOD to assess the consequences of nuclear accidents
Karlsruhe, internal KfK-report, December 1987, to be published as report KfK-4330

Title of the project no.: 3

Countermeasures to reduce the impact of accidental releases of radioactive material

Head(s) of project:

K. Burkart

Scientific staff:

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:

Parameter studies with the new program system UFOMOD in the field of emergency preparedness and emergency response.

III. Progress achieved:

Parameter studies were performed with the following main aims:

- to identify groups of the population most at risk of nonstochastic health effects
- to study the impact of different patterns of behavior of the population on the consequences of an accident
- to compare preventive evacuation with alternative strategies like sheltering followed by evacuation after passage of the plume
- to investigate the relation between intervention levels applied and benefit or extent of countermeasures.

In the calculations an early, long lasting (RL 1) and an early short release (RL 2) were assumed. Their key features are:

RL 1- in total 100% Noble gases, 2% I, 2% Cs, 1% Te etc.
mainly released in the 3rd, 4th and 9th through 13th hour.

RL 2 - in total 100% Noble gases, 8% I, 6% Cs, 4% Te etc.
uniformly released in the 2nd, 3rd and 4th hour.

A result of the calculations with source term RL 1 is shown in Fig. 1

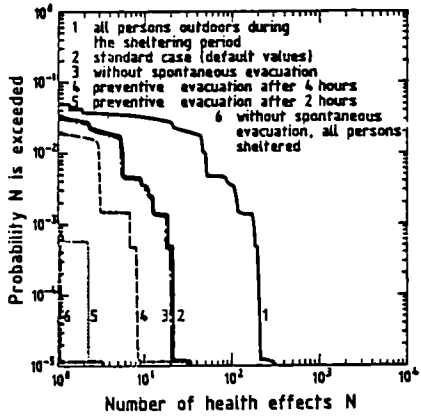

Fig. 1

CCFDs for early fatalities due to doses to the red bone marrow (hematopoietic syndrome). Release RL1.

It allows conclusions of the following kind for this type and amount of release:

- successful sheltering within 2 hours followed by evacuation after the major part of the release (here 11 hours) is sufficient to avoid early fatalities in the population
- possible early fatalities are occurring exclusively among persons remaining outdoors
- an effective alert system in a small area is much more important than vast plans for countermeasures covering large areas.

The same type of calculations has been performed with the source term RL 2. The results are leading to similar conclusions.

In view of the low emergency reference levels discussed in ICRP publication 40 and similar documents, calculations with several intervention levels between 0.5 and 6 Sv short-term dose to the red bone marrow and with the source term RL 2 were performed. The calculations show that under RL 2 conditions decreasing intervention levels of evacuation below about 4 Sv are entailing a steep increase of the evacuation area without reducing the number of nonstochastic health effects. As a preliminary result it is concluded that the main concern of emergency preparedness and emergency management should be those persons likely or potentially exposed to more than 3-4 Sv in the short term. As soon as the rescue of such persons (if any) is assured, countermeasures based on lower or more protracted radiation doses may (and will) be initiated. The previous conclusion that the number of early fatalities is most efficiently reduced by successful countermeasures in the near range is confirmed. For further clarification of this point, additional calculations without the rigid evacuation area A, routinely assumed in UFOMOD calculations, were performed. The remaining evacuation area B is exclusively determined by an intervention level of dose, subject to variation in the calculations.

The results of the parameter study are presented in Fig. 2.

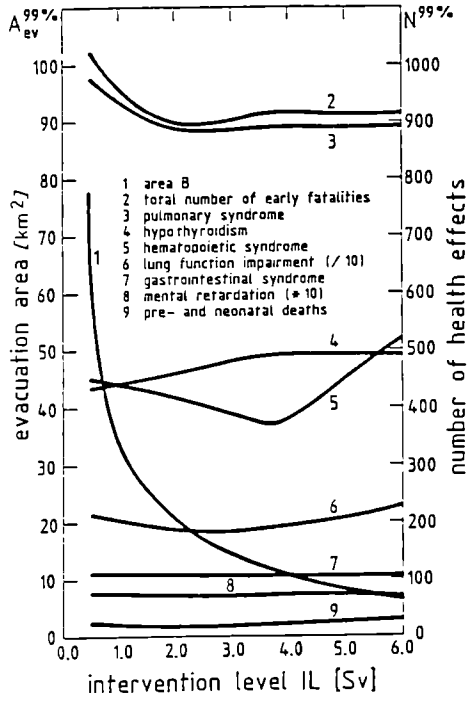

Fig 2

99%-fractile of the size of area B and

99%-fractile of nonstochastic health effects versus the value of the intervention level.
Release RL 2, no area A

At intervention levels up to about 2 Sv the total number of early fatalities decreases with increasing intervention level. Fig. 2 confirms the tendency of all conclusions mentioned above.

IV Objectives for the next reporting period

Continue parameter studies in order to gain additional insights or confirmation and to obtain a broader basis for conclusions

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

National Radiological Protection Board
Chilton
Didcot, Oxon OX11 ORQ
United Kingdom

VI. Publications

K Burkart, C Steinhauer
Ergebnisse der Deutschen Risikostudie Kernkraftwerke-Phase B:
Modellierung von Schutz- und Gegenmaßnahmen und Abschatzung ihres Umfanges
Tagungsbericht d Jahrestagung Kerntechnik '87
Karlsruhe (1987), ISSN 0720-9207

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 1987 were

- to continue uncertainty/sensitivity analyses on submodel basis using the atmospheric dispersion and deposition submodel (straight-line Gaussian plume model) of the code UFOMOD/B3.
- to repeat these analyses with revised data of parameter variations and their distributions for the trajectory models implemented in the new program system UFOMOD, version NE87/1.

III Progress achieved:

Uncertainty and sensitivity analysis tools are applied to atmospheric dispersion models of UFOMOD very effectively. Results are compared with respect to various sample sizes and different designs.

For UFOMOD/B3

- RS- and LHS-design give comparable UFOMOD uncertainty and sensitivity analysis results.
- Relative small sample sizes (1.5 times the number of model parameters) are sufficient to get statistically stable confidence and sensitivity estimates.
- Increasing sample size (n=100,200) leads to more precision in sensitivity calculations.
- The uncertainty of accident consequences in the near range (like activity concentrations, organ dose, health effects) is mainly caused by variations of thermal energy and dry deposition parameters.
- Long-term countermeasures (like relocation) and late health effects are estimated at farther distances. There uncertainties are dominated by dry and wet deposition parameters of iodine and aerosols.
- It is possible to calculate the percentage contribution of each uncertain model parameter to uncertainty in consequences (by use of the so-called 'coefficient of determination'; for details see [3]). But one has to be very careful in interpreting these coefficients of determination when correlations within the group of model parameters exist (see [5]).

Results have been presented in [1] and [2]). A detailed description of uncertainty and sensitivity results is given in [3]).

For UFOMOD, VERSION NE87/1,

before repeating the uncertainty and sensitivity analyses, a detailed discussion of the parameter variations in the atmospheric dispersion model took place together with the experts of the National Radiological Protection Board (NRPB), UK. It led to a common list of parameters to be considered and to a revision of their distribution functions and correlations (see [4]). One of the main agreements was to restrict to pure model parameters and to leave out quantities describing the source term (like thermal energy) or measured values (like wind speed or wind direction). Therefore, the list was reduced to the 20 parameters given in Figure 1, where A to F stands for diffusion category A to F.

As source term an unit release (1 Ci) of I-131 and Cs-137 in three hourly subsequent phases is chosen. The release height is assumed to be 10 meters.

The following aspects of accident consequence assessments are investigated: The concentration fields in the air near ground (1 m height) and on ground surface considering the variability of the averaged (averaged over 144 weather sequences which represent the weather of the two years 1982/83) concentration values at three distances: D1 (.875 km), D2 (4.9 km) and D3 (27 km).

PARAMETER	EXPLANATION	PARAMETER	EXPLANATION
SIGY0	HORIZONTAL DISP. FACTOR	SIGZ(B)	VERTICAL DISPERSION
SIGY0	VERTICAL DISP. FACTOR	SIGZ(C)	VERTICAL DISPERSION
HMIX	MIXING HEIGHT	SIGZ(D)	VERTICAL DISPERSION
SIGY(A)	HORIZONTAL DISPERSION	SIGZ(E)	VERTICAL DISPERSION
SIGY(B)	HORIZONTAL DISPERSION	SIGZ(F)	VERTICAL DISPERSION
SIGY(C)	HORIZONTAL DISPERSION	WP	WIND PROFILE EXPONENT
SIGY(D)	HORIZONTAL DISPERSION	VD(AER)	DRY DEPOSTION VELOCITY
SIGY(E)	HORIZONTAL DISPERSION	VD(IOD)	DRY DEPOSTION VELOCITY
SIGY(F)	HORIZONTAL DISPERSION	LD(AER)	WASHOUT COEFFICIENT
SIGZ(A)	VERTICAL DISPERSION	LD(IOD)	WASHOUT COEFFICIENT

Figure 1. Parameter list of uncertain model parameters for the atmospheric dispersion and deposition submodel of UFOMOD/NE87

The first analyses show the following results

- The restriction to pure model parameters and revised variations led to significantly smaller uncertainty bands.
- The statistical stability remarks apply as in the UFOMOD/B3-case. On the basis of n=(30,40,80) UFOMOD-runs the PRCC-sensitivity measures are calculated.
- Changes in deposition velocities and mixing height play the most important role for changes in consequences.

The analyses will be continued with all submodules of the new program system UFOMOD.

IV Objectives for the next reporting period:

The work on uncertainty analysis will continue on submodel basis using trajectory models in the new program system UFOMOD, version NE87/1 (i.e. aiming at propagation of uncertainties through the new countermeasure and dose submodels. Special investigations concerning the calculation of 'coefficients of determination' and studies with respect to changes in model parameter distributions will be included.

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] F. Fischer, "Unsicherheits- und Sensitivitätsuntersuchungen für Unfallfolgenmodelle", Jahrestagung Kerntechnik '87, Karlsruhe, June 2 - 4, 1987, Tagungsbericht des Deutschen Atomforums e.V., Bonn, p. 259 - 262

[2] F. Fischer, "Uncertainty and sensitivity analysis for computer models in accident consequence assessments", International SNS/ENS/ANS - Topical Meeting on Probabilistic Safety Assessment and Risk Management August 30 - September 4, 1987, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland in: Probabilistic Safety Assessment and Risk Management, PSA '87, Vol. III, p. 939 - 944, by Verlag TUV Rheinland GmbH, Köln, West Germany, 1987

[3] F. Fischer, J. Ehrhardt, "Analysis of uncertainties caused by the atmospheric dispersion model in accident consequence assessments with UFOMOD", Kernforschungszentrum Karlsruhe GmbH, to appear as KfK-Report No. 4262, 1988

[4] J. Pasler-Sauer, "Uncertainty analysis of the atmospheric dispersion model of UFOMOD: Selection of parameter ranges and frequency distributions", Internal Report, Kernforschungszentrum Karlsruhe GmbH, December 1987

[5] J. Raicevic, F.Fischer, "R-Square calculations for UFOMOD sensitivity studies", Internal Report, Kernforschungszentrum Karlsruhe GmbH, September 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no.: BI6-F-114-GR

Greek Atomic Energy Commission
GAEC
NRC "Democritos"
Aghia Paraskevi
GR- Attiki

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

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GAEC - NRC "Democritos"
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, Dr. N.Catsaros, Ms. E.Daoukou,
Mr. M.Christou

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) Development of a methodology for optimization of emergency response planning, and continuation of the exploration of approaches for deriving site criteria consistent with a set of general objectives, (b) Further improvement of the SHIELD-F code for calculating shielding factors of typical Greek houses, and (c) Assessment of the risk from large installations-including the impact of the Chernobyl accident in Greece - and NPP siting in the presence of large population centres.

III. Progress achieved:

1. Methodology

The principles of multiobjective optimization, risk analysis, and decision analysis are being used in the development of a procedure for the optimization of the short-term emergency response in the event of a nuclear accident.

The Patterson's method for performing double integration has been used for calculating the indoor dose-rate.

The assessment of reactor accident consequences was performed by employing CRAC.GAEC, the Greek AEC version of the CRAC2 code.

2. Results

A preliminary algorithm based on the principles of dynamic programming has been developed for the assessment of the "optimum" short-term responses to a nuclear accident. A short-term response policy determines the type of response action - evacuation, sheltering, normal activities - for each area cell around the site of the plant. The set of "optimum" responses consists of elements of the "efficient frontier" in the space of three evaluators, namely acute fatalities, latent fatalities, and cost.

The core and CPU time requirements of the SHIELD-F program have been optimized, and a 341 word array is now required instead of the 213000 words array used in the original DEP-SHIELD code.

The methodology developed for siting NPPs near large population centres has been used for the identification of acceptable sites near Athens, a city with more than three millions inhabitants. Two such areas were identified in distances below or around 50 km. These areas include two sites of historical significance, since they were proposed in the past based on different criteria.

The consequence analyses of the Greek research reactor performed, indicated that the meteorological influence on pre-

dicted consequences is not of major importance. It was also shown that in the immediate vicinity of the reactor limiting effects are the thyroid dose and thyroid latent health effects, which have practically the same magnitude for both the HEU and LEU cores.

The analysis of the radiological impact of the Chernobyl accident in Greece made apparent that the impact can be considered minor with average estimated individual doses of 0.5 mSv and 1.6 mSv for the first year after the accident and lifetime respectively.

3. Discussion

The optimization of the emergency response planning is a part of the more general approach of the multiobjective optimization with general safety goals and cost as objective functions and site, containment and plant characteristics as decision variables. This general approach will provide an analytical tool that will contribute to the rationalization of the introduction of probabilistic safety criteria - both top level and lower level - in the decision making process for the installation and operation of nuclear power plants.

The complementary site selection approach formulated, which accounts for the social radiation risk of large population centres, aimed to deal with the problems stemming from the demographic idiomorphy of Greece, where one third of the country's population is concentrated in the capital Athens. The approach is based on assessing semi-probabilistically the expected whole body collective exposure of the population centre resulting from a severe reference accident, and then comparing it to a dose corresponding to the level of social risk , for the same population sample, that is encountered historically in energy production as a whole. The result is the determination of a minimum acceptable distance from the population centre under the specific existing conditions, such as the reactor power, the population centre meteorology and wind rose, and the geographical sector of the nuclear power plant in respect to the population centre.

Finally and in relation to the objectives planned for 1987, these have been practically achieved.

IV. Objectives for the next 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.

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

VI. Publications:

A. Scientific Journals, Conferences

1. A.Vassiliou, and N.Catsaros : Indoor Dose-Rate with Point-Kernel Approximation Using a Modified NAG Double Integration Routine, Numerical Analysis Group Newsletter,1/1988.

2. N.Catsaros, and A.Vassiliou : An Assessment of the Average Shielding Factor for the Population of the Attica Basin Using the SHIELD-F Code, acc. for publication in Rad. Prot. Dosimetry. Also presented at the CEC Workshop on Consequences of an Accidental Contamination of the Urban Environment, Risø, Denmark, June 9-12, 1987.

3. J.G.Kollas : Accident Consequences Assessment and Siting Criteria Development, Joint CEC/NEA(OECD) Workshop on Recent Advances in Reactor Accident Consequence Assessment, Rome, Italy, Jan.25-29, 1988.

4. J.G.Kollas, and M.Tombrou : Modeling Nuclear Reactor Accidents - Sensitivity of the Consequences to the Meteorological Record, Proc. IASTED Intl. Symposium Modeling, Identification and Control,MIC'87, Grindelwald, Switzerland, Feb.17-20, 1987, p.438.

5. J.G.Kollas : Accidental Dose and Risk Assessment in the Immediate Vicinity of a Research Reactor, 14th Regional Congress of IRPA, Kupari, Yugoslavia, Sept.29-Oct.2, 1987.

6. J.G.Kollas : The Radiological Impact of the Chernobyl Accident in Greece, CEC Workshop on the Radiological Consequences of Chernobyl, Brussels, Belgium, Feb.3-5, 1987.

7. J.G.Kollas : An Estimation of the Chernobyl Accident Consequences in Greece - A Theoretical Approach, Symp. on the Consequences of the Chernobyl Accident in Greece, NRCPS "Demokritos", Aghia Paraskevi, Greece, Nov.19-20, 1987 (in Greek).

Title of the project no : 2

Wind Flow and Dispersion Aspects of Radiati n Risk Assessment

Head(s) of project: J.G. Bartzis

Scientific staff: J. Antoniadis, D. Assimakopoulos, N. Catsaros, G. Karras, D. Pissimanis, V. Notaridou, M. Varvayanni, A. Megaritou.

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 the computer code ADREA. The code is intendeds 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 field 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:

- Towards finalization of the ADREA-I numerical scheme
- Towards finalization of the turbulence modelling options
- Work on the 2-D reference problem (diurnal wind flow and dispersion calculations in a terrain simulating the area consisting of the Aegean sea coast, Spata plateau (10 km wide), Hymettus mountain (1024 m), Athens basin with emphasis on the sea breeze phenomena)
- Completion of the preliminary study on the wind flow field over Greece.

III. Progress achieved:

1. Methodology

The analytical work has been performed with the ADREA-I code which is under development in NRC "Demokritos" mainly within the framework of this project. ADREA-I is intended to be applicable to any terrain (complex or not) under any stability atmospheric conditions. At present the mass, momentum, energy (temperature), pollutant, turbulent kinetic energy and turbulent kinetic energy dissipation conservation equations have been already utilized.
During this period the emphasis has been given in establishing more general and reliable turbulent diffusion options, in improving ADREA-I numerical scheme and in establishing capability for complex terrain wind flow and dispersion analysis in presence of sea breeze effects.
For the flow field study in the 850 mb, the Greek territory has been divided into four parts (NW, NE, SW, SE) based on the local climatological behaviour. The analysis which coversthe years 1976-1985 is based on the synoptic maps and the sounding data of the stations, Bari (NW), Istanbul-Plovdiv (NE) and Athens -Heraklion (SE). For the flow field on the surface, the meteorological data obtained from the major station in the Greek territory have been utilized.

2. Results

Within the framework of searching for relatively simple and universal eddy viscosity/diffusivity models, a new three dimensional non isotropic model is proposed applicable to any domain complexity and any atmospheric stability conditions. The model utilizes the transport equation for turbulent kinetic energy, but introduces a new approach in effective length scale estimation based on the flow global characteristics and local atmospheric stability (1,2).
The numerical scheme has been improved by introducing an additional iterative scheme for pressure/mass correction. The NAG sparse matrix solver has been introduced as an option and utilized for pressure, energy and concentration solution.

The sea breeze circulation development prediction in case of an opposing synoptic scale wind, based on a data referring to the Alaskan Beaufort Sea Coast has been completed (3,4,5). The effects of a mountain on sea breeze and contamination patterns have been theoretically studied. A two dimensional, 1000 M, mountain range surrounded by sea and representing an idealized west-east cross section of the Athens basin has been used for the analysis (6).
A seasonal analysis has been performed on the flow field data over the Greek territory with representative months of December, March, June and September. The results are presented in terms of frequency tables of wind direction and class and wind roses/7/.

3. Discussion

The turbulent diffusion model verification studies performed during this period showed satisfactory agreement with experimental results increasing the confidence as the appropriate model in the boundary layer analysis.
The ADREA-I numerical scheme improvements have increased the maximum time step to the order of 400-4000 sec depending on the problem.
The sea breeze verification study referring to the Beaufort Alaskan sea coast, shows that the present results are in relatively good agreement with those obtained by Kozo's model. This underlines on one hand the capability of the present ADREA code to handle satisfactorily such problems and on the other hand the ability of turbulent diffusion empirical models, such as Kozo's to give reasonable results (3,4,5).
The study of the effects of a large natural barrier on sea breeze and contamination patterns shows that the anabatic wind, being reinforced by the sea breeze, result in a strong ascending air current which blocks the circulation and the contaminated air masses at the east side. Moreover, a first application of the ADREA-I code to a combined sea-mountain terrain gave reasonable results for wind field and contamination patterns.
The flow field data in the 850 mb show that the prevailing wind directions are either of the W, SW sector (December, March) or northern sector (June, September). For the latter period the SE sector is highly dominated by northerly winds due to the existence of the Cyprus thermal low. The flow field on the surface is strongly influenced by the area topography (high mountains, frequent land/sea interchange).

IV. Objectives for the next reporting period:

- Air/ground and air/sea interaction modelling
- Further work on the 2-D reference problem (diurnal wind flow and dispersion calculations in a terrain simulating the area consisting of the Aegean sea coast, Spata plateau (10 km wide), Hymettus mountain (1024 m), Athens basin) with emphasis on sea breeze penetration into the city.
- Further verification studies.

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

D. Assimakopoulos, G. Karras, D. Pissimanis, V. Notaridou
Meteorology Laboratory
University of Athens

VI. Publications:

1. Bartzis J.G., Flow Modelling in Complex Terrain for Atmospheric Applications, Intern. Symp. in Environm. Meteor. Würzburg F.R.G. Sept. 29-Oct. 1, 1987.
2. Bartzis, J.G. Turbulent Diffusion Modelling for Windflow and Dispersion Analysis. Workshop on recent Advances in Reactor Accident Consequence Assessment, Rome, Jan. 27-29, 1988.
3. Varvayanni M. and Bartzis J.G., Sea Breeze Modelling, Review DEMO Report 87/9, 1987.
4. Bartzis, J.G. and Varvayanni M., ADREA I code Development: Sea Breeze Modelling and Calculations, DEMO Report 87/8,1987.
5. Varvayanni M., and Bartzis J.G., Sea Breeze Wind Field Predictions in Atmospheric Dispersion Modelling. Workshop on Methods for Assessing the Reliab. of Environmental Transfer Models Predictions, NRCPS "Demokritos" Athens Oct. 5-9 1987.
6. 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.

Pissimanis D., Karras G., Notaridou V., Preliminary Study on the Flow Field over Greece (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. Investigation of the radon therapy centers in Edypsos and Ikaria island.
2. Further study of the indoor concentrations of radon decay products in air.
3. Investigation of the long-term consequences of the Chernobyl nuclear accident (soil pollution and root uptake by plants of agricultural importance, marine environment).

The impact of the Chernobyl accident on the Greek environment led to a number of changes in the priorities of the program, including the postponement of certain studies related with natural radioactivity.

III. Progress achieved:

1. Methodology.

1.1. A total alpha-counting procedure has been developed for the separate determination of Ra224 and Ra226 in water samples by measuring their daughters Rn220 and Rn222.

1.2. A methodology for assessment of the surface concentration of hot particles deposited after the Chernobyl accident has been developed. It made possible to detect particles of beta-emission rate $\geq$ 10 s^{-1} under conditions of specific surface emission rate of 3500 $s^{-1}m^{-2}$ and scanning velocity of 0.1 m^2 min^{-1}.

1.3. A soil sampling procedure has been developed for determination of the radioactive deposition of Cs134+Cs137. The estimated accuracy is ±10%.

In addition, high-resolution gamma-spectrometry, two total-alpha counting methods for determination of radon daughters in air and car-born scintillometry have been used in the studies reported.

2. Results

2.1. Eight radon therapy units and a number of uncaptured radon water springs have been investigated in Edypsos (Evoia island) and Aghios Kyricos/Therma (Ikaria island). The results of the measurements are summarized below:

Quantity	Ikaria	Edypsos	Units
Rn222 in water	75 - 5700	0.5 - 200	kBq m^{-3}
Ra226 in water	0.2 - 5	1.1 - 5	"
Rn222 in air	0.1 - 250*	0.1 - 5	"
	max 550**		"
Rn222 decay products in air (equil. equiv.)	max 7	max 0.4	"
Po218 in air	max 18	max 1	"
Exposition rate	15 - 60*	7 - 60*	μR h^{-1}
	max 350**	max 400**	"

* baths, ** reservoirs

These results will be presented and discussed in Ref.1.

2.2. The investigation of a region of lignite ashes deposition in Evoia island has been completed and the results have been presented in Ref.2 (the basic findings have been given in the 1986 Progress Report).

2.3. A study of the radiological impact from the operation of an experimental geothermal power plant in Milos island is in progress. The results available so far do not indicate any significant radioactive pollution of the environment.

2.4. A considerable amount of new data about the caesium deposition in Greece after the Chernobyl accident has been collected by use of soil measurements (after well-defined geometry of sampling) and car-born scintillometry. The caesium deposition pattern appears to be highly inhomogeneous, with

regional averages in the range of 1-40 kBq m^{-2} and estimated country average 9+3 kBq m^{-2} (total caesium at 5.5.1986). The correlation between caesium deposition and contamination of certain products (milk, cheese, grain) has been studied (Ref.3). Highest correlation has been observed for milk (CC=0.93), which re-confirms this product as important food indicator. The data collected have been also used to evaluate the impact on the plants during the next years (Ref.4). This is made not only by use of soil-to-plant transfer factors published by other authors, but also by direct (field) determination of these factors for certain plants, together with the measurement of important related soil parameters as pH, clay and mobile potassium content. The transfer factors determined so far vary in the range of 0.01-0.26 (dry plant to dry soil), while the wet mass concentrations of Cs134+Cs137 in the 1987 harvest plants are typically below 1 Bq kg^{-1}.

2.5. The surface density and the radionuclide content of the hot particles (HPs) in the Chernobyl fallout have been investigated (Ref.5). Two types of HPs have been identified as with regard to the gamma emitters: practically pure-ruthenium HPs and HPs of more complex non-volatile nuclide composition. Ru-HPs of activity up to 40 kBq have been detected, while the surface density of HPs of A_{tot} higher than 10 Bq (at 7.5.86) is estimated to exceed 10 per m^2 in Athens region. The probability for inhalation of HPs has been estimated by use of surface density and deposition velocity data.

2.6. The impact of the Chernobyl accident on the Greek marine environment has been further investigated (Ref.6). The influence of certain factors as biotope, temperature (metabolism rate), weathering processes and habitats-niches as well as the distribution of the radionuclides in different parts of certain marine organisms have been studied.

3. Discussion The new results from the Greek radon spas provide a further basis supporting the need for justification and optimization of the "radon therapy", in accordance with the recent ICRP statements (ICRP 50, 1987).

The new results concerning the caesium deposition in Greece during May 1986 confirm the early estimations and provide a basis for short- and long- term deposition-to-dose modelling.

The average citizen of Athens could have inhaled up to 5 HPs of initial activity $\geq$1 Bq, while for 5% of the population this activity could exceed 100 Bq. The radiological significance of these estimations depends on the intake-to-risk conversion factors for beta-emitting HPs, which seem to be not well defined so far.

There is a detectable, but of rather minor radiological significance impact of the Chernobyl accident on the Greek marine environment. It seems useful nevertheless to study the evolution of caesium concentrations in the marine biota.

IV Objectives for the next reporting period:

1. Investigation of certain regions in Northern Greece and Aegean Sea with enhanced concentrations of U238 and Th232 series radionuclides.
2. Natural radioactivity maping of the Greek soils by use of the samples collected for caesium 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 enhanced Ra226 content spa waters are continuously released in the sea.

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, NCRPS "Democritos", Aghia Paraskevi Attikis (Dr. E. Papanicolaou).

VI. Publications:

1. P.Kritidis and M.Probonas, "The Greek radon spas - hot spots of natural radioactivity in the Mediterranean area". To be presented at the International Conference on Environmental Radioactivity in the Mediterranean Area, Barcelona, Spain, May 10-13, 1988.
2. P.Kritidis and P.Angelou, "A region of lignite ashes depositions: investigation of the radiological impact". Proc. XIV Regional IRPA Congress, Kupari, Yugoslavia, Sept.29-Oct.2, 1987 (in press).
3. P.Kritidis and E.Papanicolaou, "Deposition of caesium and contamination of certain products: a correlation study". SFRP-AIRP Congress, Rome, Italy, Oct.12-13, 1987 (in pr.)
4. E.Papanicolaou and P.Kritidis, "Pollution of the Greek soils with radiocaesium and its impact on the plants: preliminary data". Proc. Nation. Congr. of Soil Science, Larissa, Greece Nov.2, 1987 (in press, in Greek).
5. P.Kritidis, N.Catsaros and M.Probonas, "Hot particles in Greece after the Chernobyl accident. Estimations on inhalation probability". Proc. Intern. Workshop "Hot Particles in the Chernobyl Fallout", Theuern, FRG, Oct.28-29, 1987 (in press).

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Publications (continued):

6. H.Florou, P.Kritidis, S.Synetos and C.Chaloulou, "Aspects radioecologiques de l'influence par les polluants radioactives au milieu marin (Grece) apres un accident nucleaire". SFRP-AIRP Congress, Rome, Italy, Oct.12-13, 1987 (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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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.

Publications (continued):

6. H.Florou, P.Kritidis, S.Synetos and C.Chaloulou, "Aspects radioecologiques de l'influence par les polluants radioactives au milieu marin (Grece) apres un accident nucleaire". SFRP-AIRP Congress, Rome, Italy, Oct.12-13, 1987 (in press).

RADIATION PROTECTION PROGRAMME

Progress Report

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Atomique, CEA
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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

M. J.F. PINEAU, CRPM/LIM, IPSN/GPMU, Saint Sylvestre, 87240 AMBAZAC

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:

Vérification des performances du Spectromètre Diffusionnel et Inertiel pour l'obtention de la répartition granulométrique de la radioactivité des descendants des gaz radioactifs naturels sur différents types d'aérosols.

Le programme des travaux de 1987 a porté principalement sur la répartition de la radioactivité des descendants du radon obtenue à partir des mesures effectuées avec le Spectromètre Diffusionnel et Inertiel (SDI 2000). On rappelle que ce dispositif a été mis au point pour étendre vers les dimensions supérieures à 0,2 µm par un impacteur les possibilités de mesures granulométriques des batteries de diffusion.

Les principaux sujets traités sont les suivants :

- utilisation en laboratoire du SDI 2000 pour caractériser la répartition de la radioactivité sur différentes distributions granulométriques d'aérosols.

- mesures préliminaires dans la chambre d'exposition de la granulométrie des aérosols radioactifs.

- élaboration d'un dispositif expérimental pour la vérification de la captation d'aérosols ultrafins ($\emptyset < 0,1$ µm) pour la partie inertielle du dispositif.

1) On a mesuré la distribution en activité des descendants du radon fixés sur différents aérosols : aérosol ultrafin produit par radiolyse, aérosol de combustion, aérosol atmosphérique. Les résultats obtenus pour les distributions des aérosols les plus fins sont très comparables à celles obtenues avec une batterie de diffusion à grilles d'usage courant.

Dans le cas des aérosols de combustions utilisés, les résultats sont sensiblement différents, en effet notre dispositif prend en compte la fraction de radioactivité fixée sur les plus grosses particules. On peut en déduire que le SDI 2000 permet, sous réserve d'une connaissance précise de la rétention éventuelle des aérosols les plus fins dans l'impacteur, la détermination de la granulométrie des descendants du radon fixés sur différents types d'aérosols.

2) Les mesures préliminaires effectuées directement dans la chambre d'exposition en présence ou non de rats montrent que la distribution de l'activité a un diamètre moyen compris entre 0,1 et 0,3 µm. On notera que pour éviter tous artefacts de mesure, la fraction libre avait été préalablement éliminée par un dispositif à grilles.

3) Dans certaines conditions expérimentales (concentration en radon très élevée) une fraction fine peut exister. Cette concentration nous a amenés à concevoir une expérience destinée à évaluer une collection éventuelle de particules inférieures à 0,1 µm dans la partie inertielle du SDI 2000. Le dispositif expérimental a été réalisé en fin d'année 1987 et devrait être opérationnel au cours de l'année 1988. Il est nécessaire de souligner que si ce piégeage a peu d'importance dans la détermination d'une granulométrie en masse, elle peut, par contre modifier sensiblement une granulométrie en nombre (cas des descendants des gaz radioactifs naturels ou artificiels).

IV. Objectives for the next reporting period:

- Poursuite de l'étalonnage de l'impacteur vis-vis des particules inférieures à 0,1 µm.

- Mesures systématiques dans la chambre d'exposition.

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

1987

Contractor:

Contract no.: BI6-F-117-IRL

University College Dublin
Belfield
IRL- Dublin 4

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:

(i) Continuation of the national radon survey.

(ii) The correlation of the acquired data with geological features and building characteristics.

(iii) The assessment of field intercomparisons of passive radon dosemeters being carried out in Brittany.

III. Progress achieved: National Indoor Radon Survey. By the end of 1987, using CR-39 closed passive alpha track detectors radon measurements had been carried out in over 1000 randomly chosen Irish dwellings. In terms of the national housing stock this represents a sampling frequency of about 1 dwelling in 850 in the State. For the etching and counting procedures used the radon sensitivity of the detectors was found to be 4.4 tracks $cm^{-2}.kBq.^{-1}m^{3}.hr^{-1}$. Participation at the 3rd CEC/OECD (NEA) radon intercomparison at the NRPB (UK) during 1987 confirmed the accuracy of this sensitivity.

The distribution of indoor radon levels in the Republic of Ireland has been found to be approximately log-normal with a median value of 31 Bq/m^3. In about 2 of the dwellings investigated the indoor radon concentration was found to be in excess of 400 Bq/m^3. As in previous survey measurements in 1986 it has again been confirmed that the main population centre in the Dublin area has low indoor radon levels with elevated levels being more prevelant in some southern and western coastal counties. Because the sampling is essentially population based the absolute number of samples taken in some counties is small. Notwithstanding this it appears that "clusters" of high indoor radon levels are present in parts of the Atlantic counties Clare and Mayo. Attention will be increasingly focussed on these and other such areas in the future as the radon national survey is now essentially completed.

Based on ICRP Publications 39 and 50 a dose conversion coefficient of 20 Bq/m^3 (radon gas) per mSv (effective dose equivalent) /year is being used for the Irish data. The survey data thus indicates that the median effective dose equivalent in Irish dwellings arising from indoor radon is about 1.5 mSv year with perhaps 2 of the population receiving doses in excess of 20 mSv/year.

Radon Detector Intercomparisons in Brittany. Intercomparisons between French (C.E.A.) and Irish passive radon detectors were made during 1986/87 under field conditions in Brittany in over 100 dwellings within the framework of a regional study of indoor radon being carried out by the Université de Bretagne Occidentale (Brest) and the Commissariat à l'Energie Atomique (Paris). Two different types of passive radon detectors, both using Kodak-Pathe LR 115 film, were used. The French type is of the open variety while the Irish type is closed. Preliminary analysis of the data obtained show that while good agreement was found in some

cases a relative variation of 40% appears to exist between the two sets of data. In general the Irish detectors gave the lower radon determinations. The reasons for this lack of agreement are being investigated. Further field intercomparisons of this type, with an Italian laboratory and perhaps other laboratories, are being considered as it is considered that this type of field intercomparison work will be a useful compliment to the more laboratory based series of intercomparisons already established by the CEC/OECD (NEA).

IV. Objectives for the next reporting period: It is intended to complete the national radon survey and make a detailed analysis of the results in terms of correlations with Irish construction characteristics and geological factors. In addition small scale regional studies in parts of the west of Ireland will be made.

It is planned to design and initiate a new phase of passive radon detector field intercomparisons with laboratories in France, in Italy and perhaps with other laboratories.

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

(i) Housing Energy Study Group
An Foras Forbartha
Waterloo Rd., Dublin

(ii) Dr. G. Tymen, Laboratoire de Physique des Aerosols et de la Radioactivité Atmosphérique. Université de Bretagne Occidentale Brest, France.

(iii) Dr. A. Rannou, Commissariat à l'Energie Atomique
IPSN-DPS-SHR-SEAPS-BP.NO.6 - Fontenay aux Roses, France.

VI. Publications:

McLaughlin, J.P. "Population Doses in Ireland". Chapter 10, Radon and its Decay Products, American Chemical Society Symposium Series No. 331. Washington D.C. 1987.

McLaughlin, J.P. and Wasiolek, P. "Radon Levels in Irish Dwellings" presented at 4th International Symposium on the Natural Radiation Environment, CEC/USDOE/LNETI, LISBON, December 1987.

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 plate-out 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: To use techniques based on electric fields and ion-generation to reduce the potential alpha energy concentration from radon daughters in room air.

III Progress achieved: Method A series of investigations on the effectiveness of electric fields, with and without unipolar ion production, in reducing the concentrations of airborne radon daughters in a $42m^3$ experimental room have been carried out. In this phase of the work the room was not sealed and was found to have typically a natural air exchange rate with its surroundings of about 1 hr^{-1} depending on weather changes and building usage patterns. By the use of a combination of fine steel meshes and membrane filters it was possible to determine the air concentrations of RaA (Po-218), RaB (Pb-214) and RaC (Bi-214) in both the attached and unattached states. Radon levels required were produced in the room from a 5MBq dry radium source. Electric fields and unipolar ions in the room were produced by means of a + 30kV power supply connected to a single electrode mounted in the middle of the room about 2m above the floor. The electrode could be shielded to produce a static field or unshielded to produce unipolar ions of either polarity in addition to the field.

Results In the case of static field generation of both polarities no significant reduction in radon daughter levels could be detected with shielded electrode potentials up to 30kV. This is not surprising as in the arrangement used a strong field gradient only exists close to the electrode where very localised radon daughter depletion may occur. Throughout the bulk of the room air volume where the field gradient is low the field removal is also low. In the case of static fields with unipolar ion production by corona discharge at the unshielded electrode very marked reductions in radon daughter concentrations were achieved. This increased removal efficiency is interpreted as being due to a modification by the ions of the field gradient within the room to the extent that effective radon daughter removal throughout the bulk of the airspace could be acheived. In this form of air treatment the radon daughter reductions were quite significant and took place very rapidly. Fields with positive ion production were about a factor of two more efficient than fields with negative ion production. The table that follows shows the results obtained after 10 minutes of air treatment on air with a radon concentration of 2500 Bq/m^3 subjected to a field and unipolar ions from a corona point at 29kV. The radon daughter activities achieved in each case are expressed as a percentage of their initial values before

treatment.

Radon Daughter	Positive Ions % of Initial Value	Negative Ions % of Initial Value
RaA	30	52
RaB	22	51
RaC	19	46

For field and positive ion production the typical reduction of the working level value was to about 25% of its initial value and to about 50% in the case of negative ion production conditions.

Investigations were also made to determine the separate effect of unipolar ion production on attached and on unattached radon daughters. It was found while marked reductions in absolute values of radon daughters could be achieved that the relative fraction of unattached daughters was greater in the treated air than in the untreated air. This is shown in the table that follows which given the attached to unattached ratio before and after treatment.

	Attached/Unattached Ratio	
Radon Daughter	Before	After
RaA	0.73 : 0.27	0.23 : 0.77
RaB	0.93 : 0.07	0.43 : 0.57
RaC	0.92 : 0.08	0.40 : 0.60

It is apparent that this particular form of air treatment (field combined with unipolar ions) has a greater effect on the attached activity than on the unattached activity. In view of the significant contribution to lung dose which appears to be made by unattached activity a closer investigation of this aspect of the air treatment is required. The investigations carried out so far show that airborne radon daughter levels and lung dose may be reduced significantly by these techniques. The optimisation of this form of air treatment and the testing of practical devices for households requires further study.

IV. Objectives for the next reporting period: The optimisation of the combined electric field and unipolar ion production radon daughter removal technique in the experimental room. In addition practical devices for use in homes based on this air treatment approach will be tested.

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

Laboratory of Applied Physics 1
Technical University of Denmark
Lyngby, Denmark
(Dr. N. Jonassen)

VI. Publications: There have been no publications on this project material during the reporting period.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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
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118 rue de la Tombe Issoire
F-75014 Paris

Mr. 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 N°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

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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.

En effet, dans le but d'une gestion des nuisances, on ne peut plus privilégier, à priori, l'une ou l'autre de ces sources d'exposition. Il faut donc au préalable les évaluer dans un contexte réel.

Par ailleurs, une reflexion est nécessaire sur la possibilité d'aggréger, pour le décideur, des évaluations du risque obtenues en fonctionnement normal des installations à celles estimées à la suite d'un accident.

II. OBJECTIVES OF THE REPORTING PERIOD

L'année 1987 a vu progresser, dans les structures mises en place en 1986, l'étude de deux sources de nuisances (radioactives ou non) qui prises isolément sont considérées comme peu polluantes mais dont la multiplicité augmente les effets :

. le chauffage domestique et tertiaire (charbon, fuel, bois, gaz)
. l'habitation (radon par exemple) et les activités domestiques qui y sont pratiquées (produits d'entretien de la maison, bricolage, hygiène corporel, ...).

Par ailleurs, afin de compléter les résultats obtenus (cycles énergétiques) dans le secteur industriel, la recherche de données d'émission relatives à d'autres industries (sidérurgie, cimenteries, chimie, agro-alimentaire,...) a été poursuivie.

III. PROGRESS ACHIEVED

La banque de données nécessaires à l'évaluation de la pollution externe due au chauffage a été complétée par la consommation au niveau départemental, en différents combustibles (minéraux solides, fuel lourd, gaz, GPL, bois, résidus urbains) du secteur résidentiel, tertiaire et industriel. Par ailleurs, la consommation du secteur résidentiel a été détaillée :

. chauffage central collectif
. chauffage central individuel en immeuble collectif
. chauffage central individuel en maison individuelle
. appareils indépendants de chauffage
. appareils indépendants d'eau chaude
. appareils indépendants de cuisson

Les facteurs d'émission en SO_2, NOx, CO_2 et poussières pour chacun des combustibles cités ci-dessus ont été soit recherchés dans la littérature, soit évalués par des expérimentations adéquates. Les émissions de composés organiques volatils et d'hydrocarbures polycycliques aromatiques issues de la combustion du bois ont été évaluées expérimentalement.

Un modèle de dispersion atmosphérique multisources simplifié est en cours d'élaboration.

Une étude statistique sur la consommation moyenne annuelle en produits domestiques d'usage courant par la population du Sud-Est de la France a montré que :

. la composition des produits étaient jalousement gardée par les fabricants et que celles notées sur les produits sont soit inexistantes, soit incomplètes dans le meilleurs des cas.
. pour une famille de produits, tous les produits n'étaient pas très différents et qu'il suffisait d'en étudier un échantillon pour connaître les compositions de base.
. les indices d'achat au niveau départemental et au niveau de la région étudiée sont assez peu différents du niveau national (indice 100). Ils varient dans l'intervalle 95-105.

Une analyse bibliographique a fait le point des connaissances sur la pollution à l'intérieur des locaux et a montré que si on dispose de suffisamment de données pour certains polluants (SO_2, CO_2, formaldéhyde) il est nécessaire de poursuivre d'autres études tant au niveau des concentrations ambiantes (radon, composés organiques volatils) que des effets sur la santé en exposition chronique (les polluants en général).

Un recensement des émissions industrielles disponibles au niveau national (Ministère de l'Environnement) a mis en évidence les secteurs industriels pour lesquels une évaluation de l' exposition pour le public pourra être estimée.

Une analyse de l'impact de la décentralisation sur les systèmes décisionnels ainsi que l'étude de l'optimisation de l'utilisation des données de sécurité et d'environnement a été commencée.

IV- OBJECTIVES FOR THE NEXT REPORTING PERIOD

1- Une fois terminées les différentes études statistiques et expérimentales destinées à caractériser les sources d'exposition présentées ci-dessus, les évaluations des nuisances résultantes pour la population du Sud-Est de la France seront effectuées.

2- Afin de mettre en perspective ces nuisances avec celles issues de la production d'énergie dans la région considérée, les évaluations précédemment réalisées (année de référence: 1982) seront réactualisées.

3- L'étude de faisabilité de l'évaluation des nuisances issues des émissions de certaines petites et moyennes industries commencée en 1987 sera poursuivie car l'obtention des données d'émission présente souvent une importante difficulté.

4- Une reflexion portera sur la possibilité d'aggréger, pour le décideur, des évaluations du risque obtenues en fonctionnement normal des installations à celles estimées à la suite d'un accident.

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

- Coulon. R., Aigueperse. J., Anguenot. F. - Etude comparative sous les deux aspects radioactif et chimique de l'impact sur la population des industries conventionnelles et nucléaires (rapport final du contrat BIO.F.320.81.F) Institut de Protection et de Sureté Nucléaire, Programme Grand Delta, 1987.

- Meme rapport. Edition C.C.E. en anglais, en cours
- - Coulon. R., Aigueperse. J., Anguenot. F. - Etude comparative sous les deux aspects radioactif et chimique de l'impact sur la population des industries conventionnelles et nucléaires. Pollution Atmosphérique n°115, 264-265, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.:

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) To acquire a high sampling rate multi-stage cascade impactor capable of quantitative measurements of particle size distributions up to about 100 um. To carry out trials locally and initiate a programme of measurements using the device at suitable sites on the Cumbrian coast.

(b) To measure the aspiration efficiency as a function of particle size of a high volume air sampler at present in operation in west Cumbria. To use the results to interpret activity concentration measurements obtained from this air sampler.

III Progress achieved

The nuclear fuel reprocessing plant at Sellafield on the north-west coast of England discharges radionuclides both to sea and to the atmosphere. Previous investigations of airborne radionuclides in the environment around Sellafield indicate that there are two characteristic components to the radioactive content of the aerosol:

i) Particles with an AMAD (Activity Median Aerodynamic Diameter) in the range 1 to 3 um, with which predominantly fission products are associated. This component is believed to originate from discharges directly to atmosphere.

ii) Particles with a much larger AMAD (> 10 um), with which predominantly actinides (plutonium, ^{241}Am) are associated. This component is believed to be of marine origin. Assessment of exposure of the public has to take account of inhalation of these particles. They also provide a pathway by which actinides originally discharged to sea are transferred to the terrestrial environment. Because these particles have such large aerodynamic diameters, equipment in current use is unlikely to obtain representative samples of this component.

The immediate objectives of the project are therefore to characterise this component of the aerosol and evaluate the collection efficiency of the existing sampler for it.

There has been limited progress on this project, primarily because of the need to redirect effort onto Chernobyl- and radon-associated problems, but the requirements of the former are now gradually decreasing. Furthermore, scientists at the Universities of Essex and Lancaster have recently proposed to carry out a similar project, and following discussions it has been agreed that if they are enabled to proceed, we shall collaborate with them, to speed development of suitable instrumentation and conduct a more comprehensive measurement programme than our resources would permit.

a) Determination of the activity concentration of the aerosol as a function of particle aerodynamic diameter.

There are three major difficulties specific to this situation to be overcome: collecting representative samples of such large particles; protecting the instrument from the weather, especially rain; and sampling a sufficiently large volume of air. Since the limit of detection of the radionuclides of interest with the technique available is about 2 mBq, and air concentrations are of the order of 10^{-6} Bq m^{-3}, the volume sampled and the sampling rate need to be of the order of 10^{4} m^{3}, and 1 m^{3} min^{-1} respectively.

Two approaches which have been used elsewhere for studies of large particles (but not for radioactive particles, nor under the hostile weather conditions expected) are being investigated. Both should in principle collect representative fractions, classify them, and have adequate sampling rates. Both would, however, require modification for our purposes: the impaction surfaces could quickly become overloaded; excessive moisture (rain, fog) could wash deposits from the surfaces; the activity in a particular size range would be determined from the difference between two samples, which could lead to large errors at the low concentrations involved.

i) The rotary impactor. This consists of impaction plates on rotating arms which collect particles above sizes determined by the plate dimensions and speed of rotation. As its construction is relatively simple, it is proposed to make one to determine the limits of its applicability. Being relatively compact it could be tested in a wind-tunnel and at least be used as a field standard for other instruments.

ii) The Wide Range Aerosol Classifier (WRAC). The large inlet (0.6 m diameter) through which a very high flow (40 m^3 min^{-1}) passes, collects particles up to 100 um efficiently under most conditions. Parallel impactors sample from this flow at 1.5 m^3 min^{-1} each. Although bulky and expensive, it appears to come closest to meeting our requirements, and also has the merit of being a standard instrument, as it is used by groups in the USA and FRG. Enquiries were made with a view to acquiring a similar instrument from the FRG, but this approach proved to be beyond the resources of the project. It is therefore planned as a first step to construct a sampler housing, based on the WRAC inlet. This will be used with a standard air sampler to obtain representative samples of total airborne activity, and means of separating the particles by aerodynamic diameter will be investigated.

b) Collection characteristics of the existing sampler.

The air sampler in current use consists of an upward-facing open face filter through which air is drawn at about 1 m^3 min^{-1}, housed in a wooden shelter 1.3 m x 1.3 m x 2 m high, with a 0.2 m x 0.2 m inlet in each side. Because of its size, it is impractical to measure its collection efficiency in a wind-tunnel. It is therefore planned to compare samples of the ambient aerosol (augmented if necessary) taken inside the shelter, with simultaneous samples taken outside with a rotary impactor and a sampler in the high-efficiency inlet described above. To this end a copy of the sampler housing has been constructed and set up at Chilton. The collected material will be resuspended in the laboratory using the Small-Scale Powder Disperser (TSI Inc.) recently acquired, and measured using the NRPB's Aerodynamic Particle Sizer (APS). For this purpose a recently-announced upgrade for the APS, which raises its upper limit from 15 um to 30 um will be obtained.

IV. Objectives for the next reporting period:

i) To construct and test a rotary impactor.

ii) To construct a high efficiency air sampler based on the inlet to the WRAC; and to investigate methods for classifying particles collected by it.

iii) To determine the collection efficiency of the existing sampler.

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

Dr C N Hewitt, Dr M Kelly, Department of Environmental Science, University of Lancaster, UK.

Dr R M Harrison, Institute of Aerosol Science, University of Essex, UK.

Dr J Vincent, Institute of Occupational Medicine, Roxburgh Place, Edinburgh, UK.

VI. Publications:

None.

Title of the project no.: 2

Study of methods for remedying and preventing high radon levels in dwellings.

Head(s) of project:

A C James

Scientific staff:

K D Cliff, J C H Miles, J C Strong, P R Lomas, R A Algar

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 select and obtain phosphate sand with sufficiently high radon emanating power and physical stability to serve as infill soil under experimental floor structures. To study methods of reducing radon concentrations in occupied dwellings and workplaces.

III Progress achieved

The mass-size distributions of four samples of phosphate sand from different suppliers were measured. Two of these samples were found to be unsuitable on structural grounds, having 10% of mass in the silt and clay size-fraction of particle diameter less that 60 μm. Such material is likely to block bore-holes drilled to drain the test site (which was described in the previous report). The two other samples were found to have similar emanating power and air permeability exceeding 2 10^{-11} m^2, at which theoretical studies indicate that pressure-driven flow of soil gas should be adequately high. One of these samples was selected on grounds of lower cost. The quantity required to fill the excavated site is being delivered.

The mechanism of radon entry into a single-storey dwelling with a large area of timbered floor and naturally ventilated underfloor space has been investigated. Even though the floor in each room was covered with normal materials, it was found that radon-laden air from the underfloor space entered at a rate of 1 m^3 h^{-1} per m^2 of floor. Increasing the open area of grilles built to ventilate the underfloor space naturally with outdoor air had no effect on radon ingress. However, mechanical underfloor ventilation by means of a 60 W fan reduced the indoor radon concentration eight-fold within two hours, equivalent to 1.4 air changes per hour indoor ventilation for a constant source. In order to achieve passive control of radon entry, an impermeable membrane is being laid and sealed to the walls before reinstating normal coverings.

Preliminary tests of a device that employs positive space charging and enhanced convection to increase the plate-out of unattached radon daughters to room surfaces (Maher E F et al. Effective removal of airborne Rn-222 decay products inside buildings. Hlth Phys. 53, 351-356, 1987) have been carried out. It is not proposed to use such devices for long-term control of indoor radon but they may have an interim role to alleviate very high concentrations while a permanent remedy is sought. A fivefold reduction in the concentration of potential alpha-energy was achieved in the NRPB large radon chamber at a ventilation rate of less than 0.1 h^{-1}. The effect of this device on the unattached fraction and activity-size distribution of the radon daughter aerosol, and thus its effectiveness in reducing lung dose, is being studied; it is also being tested in a dwelling under normal household conditions.

A duty visit was made (by Cliff, NRPB with Dr Warren, Building Research Establishment) to the Building, Ventilation and Indoor Air Quality Group, Lawrence Berkeley Laboratory, University of California (Drs A V Nero and R G Sextro). Remedial work on dwellings in Pennsylvania and New Jersey was inspected and discussed with the scientists concerned, from LBL, the University of Princeton, Oak Ridge National Laboratory, the US Environmental Protection Agency and their consultants in the field.

IV. Objectives for the next 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 special floors designed to prevent radon entry.

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

The Building Research Establishment, Garston, Watford, WD2 7JR.

VI. Publications:

Remedial measures in a house with high radon levels. K D Cliff, A D Wrixon, J C H Miles and P R Lomas. IN: Radon and its Decay Products: Occurrence, Properties, and Health Effects. ACS Symposium Series 331, P K Hopke, Ed. American Chemical Society, Washington DC, pp 536-559 (1987).

Radon remedies in dwellings. K D Cliff. Radiol. Prot. Bull. 79, 11-14 (1987).

Radon control and remedies in buildings. K D Cliff, J R Britten, D W Dixon, P H Gardner and P A T Richardson. Presented at the IV International Symposium on the Natural Radiation Environment, Lisboa, Portugal, December 7-11, 1987.

RADIATION PROTECTION PROGRAMME
Progress Report

1987

Contractor: Contract no.: BI6-F-119-F

CEN-IPSN de Fontenay-aux-Roses
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Head(s) of research team(s) [name(s) and address(es)]:

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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° : 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. 1987

1. Quantification des impacts psycho-sociaux: méthodologie et illustration dans diverses études de cas.
2. Conséquences économiques: revue des différentes méthodes d'évaluation et application au cas d'une zone urbaine fortement industrialisée.

III. ETAT D'AVANCEMENT

1. La quantification des impacts psycho-sociaux d'une évacuation passe d'abord par l'observation et l'analyse de diverses situations accidentelles ayant conduit à des évacuations spontanées ou plus ou moins organisées. Si l'occurrence de telles situations est finalement assez élevée, leur description précise (chronologie détaillée et analyse des réactions des populations présentées dans des documents synthétiques) est assez rare. On peut trouver, comme par exemple dans "Accident at Three-Mile Island. The human dimensions." Colorado Westview Press, les deux points qui sont susceptibles de quantification : a) les opinions des résidents sur leur état d'esprit dans une telle situation et sur leurs sentiments vis à vis de l'organisation des secours et de la gestion de la crise, b) leurs comportements tout au long de la crise. Les méthodes employées procèdent par entretiens non directifs et par enquêtes auprès des résidents ainsi que par l'observation de l'emploi qui est fait des moyens disponibles. La quantification se base alors sur la statistique. Cette rubrique des impacts psycho-sociaux sera détaillée dans le rapport de fin d'étude.

2. Les méthodes d'évaluation des consequences economiques ont ete présentées dans une communication faite lors du "Workshop on consequences of an accidental contamination of the urban environment" organisé par les Communautes à Roskilde en Juin 1987. Le texte doit être publié en 1988 dans la revue Radiation Protection Dosimetry. On peut considérer que la perte économique associee à la cessation d'activité dans une zone urbaine industrialisée de 4000 0 personnes évacuée totalement durant un mois sans dégradation de l'appareil productif est de l'ordre de 2 milliards de Francs. Il faut souligner l'importance de la duree de l'evacuation qui conduit à choisir une méthode d'évaluation plutôt qu'une autre et le rôle des aides et subventions financières qui peuvent modifier considérablement l'impact local ou régional d'une evacuation sans changer bien evidemment le total de la perte.

IV. OBJECTIFS DE LA PROCHAINE PERIODE : Jan.-Juin 1988

Les six derniers mois du contrat seront consacres à la synthese des points de vue économiques et psycho-sociaux qui sera realisée au cours de la rédaction du rapport final.

V. AUTRE GROUPE DE RECHERCHE COLLABORANT AU PROJET

VI. REFERENCES

Assouline M., Bastien M.C., Brenot J., Dumas M., Parmentier N.
Economic consequences of evacuation in industrialised urban areas.
Radiation Protection Dosimetry (to be published).

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 Bouisset, 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 radionuclides deposited ;
- assessment of protection factors, i.e. dose reducing ratio if sheltering of people in dwelling is planned

II. Objectives for the reporting period:

Sampling a set of dwelling in statistical data base and calculation of protection factors- Application for some French sites

Intercomparison with Gesellschaft für Strahlen-und-Umweltforschung calculations and comparison with experimental results

III. Progress achieved:

1. Methodology

1 - Housing characteristics and sampling of buildings

A statistical survey is conducted on housing located around French nuclear power plant. Four housing types are considered : old and recent single family, old and recent multifamily. Within each site for each housing type the housing characteristics are collected.

For each of the four basic categories an average building is established. It is defined as a parallelepiped, whose ground surface is determined and for which the width between two main facades has been best evaluated during cadastral studies. Triangle roofs are represented by a section of parallelepiped whose exposed surface are equivalent. The volumetric mode is completed with evaluation of the densities of interior partition and the room dimensions.

To describe the diverse variant characteristics of a site the technologies and materials liable to have been used in the construction of the vertical walls and the roof are considered. Single family can be contiguous or not and they can have one to three floors.The probability of each variant is calculated and all variants which have a probability greater than 0.01 are considered in the sampling of representative buildings for each sites.

2 - Experiments

To verify the method of calculation f the mean kerma rates in an apartment or a building which is used in our computer code, a small house have been built near a cesium 137 source located in natural environment at Cadarache. This model of house is defined by actual parameters : wall and roof technologies, inhabitable surface and volume, aperture surface.

The choosen wall material is bondstone which is frequently used in recent building and which represents one of the most unfavourable wall technologies. The tile which is the more frequent roofing material is considered.

Four experiments have been made :

1. roof and front are exposed to radiation. The house has a door and a window on front (surface 2.75 m2), and one room (inhabitable surface 12 m2) ;
2. same conditions that in experiment 1., but there is only a door on front (surface 1.75 m2) ;
3. same conditions that in experiment 2., but the room is divided in three small volumes by a partition (inhabitable surface 4.35 m2, 4.5 m2 and 3 m2) ;
4. same conditions that in experiment 3., but the front is raised up to shield the roof.

Dosimetric measurements with TLD dosemeters are made outside and inside the house. In the house the dosemeter locations describe all the inhabitable volume : 48 locations in experiments 1 and 2, and 52 locations in experiments 3 et 4.

Three calculations are made for each experiment : kerma at dosemeter locations, average kerma in the house with actual door and window, average kerma in the house with door and window defined by their surface probability.

2. Results

1 - Statistical survey of housing characteristics and sampling of representative building

Statistical survey of housing characteristics made by "**Association de Pratique Architecturale et Urbanistique**" is finished in December 1987. The housing conditions in the surrounding of all the French nuclear power plant sites have been collected. Methodology and first results have been presented at RISO Workshop (June 9-12, 1987).

2 - Experiments

Two campaigns have been made :
1) In September 1987,four experiments with cesium 137 source (760 Ci) and 2) in October 1987, two experiments (with and without window) with a cobalt 60 source (117 mCi) for spectrometric measurements in collaboration with GSF.

Results of the first campaign are summarized by the two following tables :

a) Kerma rates outside in front of the house ($mGy.h^{-1}$)

height above ground floor (cm)	measured	calculated
50	45.2 ± 3.8	45.4 ± 3.7
100	49.5 ± 4.2	50.9 ± 3.1
150	53.5 ± 3.8	56.4 ± 4.8
220	62.5 ± 1.4	60.5 ± 3.7

b) Average kerma rates in the house ($mGy.h^{-1}$)

Experiment	TLD locations		Model with door and window	
	measured	calculated	actual	in probability
1	15.3	14.5	14.2(14.6)	14.1(14.5)
2	12.9	12.6	12.0(12.3)	12.5(12.8)
3	12.5	12.8	17.7(12.6)	18.4(13.1)
4	10.0	10.1	13.9(9.9)	15.1(10.8)

Differences for average kerma rates between measurements and calculation appear only in experiments 3 and 4. Average kerma depends on room inhabitable surface but also on height between floor and ceiling. A corrector factor has been defined and applied (number between brackets).

3. Discussion

In an apartment the main rooms which have inhabitable surfaces in the range of 9 to 15 m2, are more frequent than small or large rooms. Then the influence of the corrector factor on average kerma rates in apartment or dwelling is relatively low. However it must be studied for all the representative building sampled in the surrounding of each site.

IV Objectives for the next reporting period:

- Dose data base for immersion in cloud of radionuclide releases in open air.
- Collaboration with GSF will be continued.
- 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.
- Our computer code will be developed for deposit on ground and open air building surfaces.

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 Chemin des Maraichers - 31062 Toulouse Cedex

A.P.R.A.U. (Association de Pratique et de Recherche Architecturale & Urbanistique N° 5) - 58 Avenue Salvador Allende - 92000 Nanterre.

VI. Publications:

J. LE GRAND, J.C. CROIZE, T. de DORLODOT and Y. ROUX
Statistical Survey of the housing characteristics and evaluation of shielding factors in the surroundings of French nuclear sites.
Workshop on Consequence of an accidental contamination of the urban environment, RISO, June 9-12, 1987.

Title of the project no.:

EVALUATION DES DOSES AUX POPULATIONS OU A LA SUITE D'UN ACCIDENT ENTRAINANT DES REJETS DE GAZ ET D'AEROSOLS RADIOACTIFS DANS L'ATMOSPHERE.

Head(s) of project:

Docteur N. PARMENTIER
Service d'Etudes Appliquées de Protection Sanitaire
B.P. n° 06
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 à longue distance. Les calculs d'incertitudes et les calculs de conséquences radiologiques sont également inclus dans ce projet.

II. Objectives for the reporting period:

Cette période a eu pour objectif de développer un modèle de diffusion-advection tridimensionnel permettant de prendre en compte les effets du relief. Le modèle a été établi à partir des études théoriques faites sur la résolution de l'équation en 1986 et décrit dans le rapport CEA-R-5364. La plus grande partie des travaux effectués durant cette période a consisté à concrétiser ces études théoriques par la réalisation d'un code de calcul numérique.

DISCUSSION :

Nous avons développé ce code de calcul de transport de particules qui peut apparaître sophistiqué mais qui peut traiter d'autres problèmes que ceux du transfert atmosphérique, ce qui explique la complexité notamment des conditions aux limites. Il apparait cependant que la qualification et la validation de ce code de calcul, qui s'avère exploitable à courte, moyenne et longue distance, seront plus longues et délicates que prévu.

III. Progress achieved:

Les résultats de l'avancement des travaux relatifs à cette période se sont en totalité traduits par l'écriture d'un programme de calcul dont les bases mathématiques et la méthodologie ont été décrits dans le précédent rapport d'avancement. Le programme a été écrit en langage Fortran et permet de faire des calculs de diffusion-convection, mono, bi ou tri dimensionnels. Ce code de calcul comprend trois parties :

La première partie a pour objectif la description physique du milieu mono, bi ou tri dimensionnel. Le milieu tri dimensionnel est défini par un maillage régulier dont un élément est appelé "pavé". Chaque pavé est associé à un ensemble d'informations :

- un vecteur (module et direction) définissant la convection.
- un tenseur de diffusion anisotropique (coefficients de diffusion en x, y et z).
- un coefficient d'absorption volumique.
- des conditions aux limites généralisées sur chaque face du pavé, c'est-à-dire munies, entre autres, des possibilités suivantes :
  - . réflexion totale
  - . transmission avec albedo (une particule heurtant la face du pavé a une probabilité r de la traverser et une probabilité 1-r d'être réfléchie.
  - . réflexion avec perte d'énergie, sans perte d'énergie.
  - . absorbption totale ou partielle par la face du pavé.

La seconde partie du code de calcul a pour objectif de calculer la distribution spatio-temporelle des particules émises sur le domaine décrit et muni des propriétés décrites ci-dessus. La précision des calculs est fonction du nombre de particules émises à partir d'un point du domaine mentionné par l'utilisateur.

Les résultats obtenus consistent en une série de valeurs décrivant la fraction du nombre de particules émises et présentes dans chacun des pavés, en fonction du temps.

La troisième partie de ce code de calcul permet d'obtenir une représentation graphique de ces distributions.

Jusqu'à présent ce code a éte comparé à des codes de calcul de type panache gaussien, afin de tester sa robustesse numérique. Les résultats se sont montres tres favorables puisque les écarts entre ces deux types de modelisations (analytique et numerique) n'ont jamais excédé 5 .

IV. Objectives for the next reporting period:

La prochaine période contractuelle (Juillet 1988 à Juillet 1989) permettra le développement d'une méthode d'analyse d'incertitude permettant de remplacer le concept de dose moyenne par celui de distribution de dose. Une grande partie des paramètres utiles dans les précédentes études étant munie d'une importante incertitude, l'objectif de cette étude sera de pouvoir établir une fourchette de valeurs de dose la plus réaliste possible.

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

VI. Publications: NON

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)].

Dr. J. Porstendörfer
Isotopenlaboratorium
Georg-August-Universität
Burckhardtweg 2
D-3400 Göttingen

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

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 distributions, 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 nm and 10000 nm in houses and in the open air. Model calculations have to be performed to determine specific parameters (plateout rates, attachment rates, etc.) and the radiation exposure.

II. Objectives for the reporting period:

The influence of the measured equilibrium factor F and the free fraction f_p on the radiation exposure has to be calculated using different dosimetric models. Measurements of activity size distributions in closed rooms under various conditions (influence of aerosol sources and ventilation rates) will be performed. Measurements with the Berner impactor and screens in series are planned to determine the size distribution in the diameter size range smaller than 100 nm with higher accuracy, and the efficiency curves of this new device have to be determined with monodisperse test aerosols.

Intercomparison measurements will be performed with the Nuclear Physics Laboratory of Ghent.

III. Progress achieved:

1. Methodology

Activity size distributions of the shortlived radon decay products ^{218}Po, ^{214}Pb and $^{214}Bi/^{214}Po$ were measured in dwellings with a low pressure cascade impactor (type: Berner) and a device of high volume screen diffusion batteries under various conditions. Several activity size distribution measurements were carried out in five rooms without additional aerosol sources at higher radon levels of 200 - 1700 Bqm^{-3}. All rooms were closed with low or moderate ventilation rates ($v < 1.0\ h^{-1}$) and the aerosol particle concentrations Z varied between 2000 and 16000 cm^{-3}. The influence of aerosol particles of different additional aerosol sources (Z up to $30*10^4\ cm^{-3}$) on the activity size distribution was studied in three rooms with the diffusion battery technique.
Activity size distributions are received from the size fractionated activities, measured with the low pressure impactor and the diffusion batteries by comparing the measured activities with the simulated ones. The simulated values were determined using the efficiency curves of the impactor and the penetration curves of the diffusion batteries, respectively, whereby the true size distributions are approximated by a sum of lognormal distributions.

The dynamics of radon daughters in realistic indoor environment (deposition rates, unattached fraction, etc.) has been investigated experimentally by the laboratories of Ghent (Vanmarcke - Raes) and Göttingen (Reineking - Porstendörfer) using quite different methodologies. These methodologies were compared by means of joint measurements performed in an old house with elevated radon concentrations, on the average 310 Bqm^{-3}. Twentyone measurements were carried out on 4 different days.

2. Results

In low ventilated rooms without additional aerosol sources the activity size distributions can be described by one lognormal distribution (accumulation mode) with average activity median aerodynamic diameters (AMAD) of 188 - 234 nm (range 115 - 370 nm) and average geometric standard deviations σ_g of 2.0 - 2.5 (range 1.4 - 4.3). The mean diffusion equivalent diameter of the unattached activity of ^{218}Po was determined to be 1.2 nm (range 0.5 - 2.0 nm). Additional aerosol sources (electric motor, candle light) sometimes yield to a second maximum of the attached fraction in the diameter size range of 10 - 100 nm (nuclei mode) or the accumulation mode can be shifted to greater sizes (cigarette smoke: AMAD = 300 nm).

The obtained total activity concentrations during the intercomparison measurements of the groups of Göttingen and Ghent broadly agree, with differences up to 20 %. From the ratio of the measured attached and unattached ^{218}Po concentration Reineking calculates a mean attachment rate of 112 h^{-1}, whereas Vanmarcke calculates a value about half of those from the measured inactive particle size distribution. The agreement, as to the deposition rate of the attached radon daughters is better. Vanmarcke calculates a mean value of 0.22 h^{-1} and Reineking a mean value of 0.30 h^{-1}. Reineking determined deposition rates of the unattached radon daughters between 43 h^{-1} and 146 h^{-1} with an average value of 74 h^{-1}.

Vanmarcke computes (20 ± 10) h^{-1}. The unattached fraction in the room is less than 10 % due to the high particle concentrations. The values of Reineking are about half of those of Vanmarcke. The agreement for the equilibrium factor is good, with differences less than 20 %. The equilibrium factor in the room ranged from 0.4, without aerosol sources, to 0.8, after smoking some cigarettes.

3. Discussion

The measured indoor activity size distribution of aerosol attached shortlived radon daughters agree with our former high - volume impactor studies and with results published in the literature (Becker et al., Sinclair et al., Knutson et al.). The diffusion equivalent median diameter of the unattached ^{218}Po activity was determined to be 1.2 nm.

The intercomparison measurement with the group of Ghent yielded to the result that the measured total radon daughter concentrations agree, but the obtained attachment rate, the deposition rate of the unattached fraction differ significantly, while the results for the deposition rate of the attached daughters and the equilibrium factor agree.

First test measurements with a combination of the cascade impactor and screens in serie were performed. Instead of the intended calibration measurements of this device, we started to calibrate the low pressure impactor with monodisperse aerosol particles in the diameter size range smaller than 100 nm by varying the pressure of the last impactor stages.

The influence of the measured free fraction f_p and the equilibrium factor F on the radiation exposure, expressed by the effective dose equivalent H_E per radon exposure $c_o t$, was studied using different dosimetric models. The conversion factor as to the JAMES-BIRCHALL model can drastically change in room air (3 - 7 $nSvBq^{-1}m^3h^{-1}$), whereas the JACOBI - EISFELD model gives a fairly constant value of 5 $nSvBq^{-1}m^3 h^{-1}$, independent of the aerosol parameter.

IV. Objectives for the next 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 atmophere 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 activties will be determined in the outdoor environment.
Intercomparison measurements with other groups will be repeated in a house with higher unattached fractions.

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

VI. Publications:

Porstendörfer, J. "Indoor Radon Exposure in the Federal Republic of Germany" presented at the Second International Specialty Conference on Indoor Radon, New Jersey, USA, April 6-10, 1987

Reineking, A., Becker, K.H., Porstendörfer, J. "Measurements of Activity Size Distributions of the Short-Lived Radon Daughters in the Indoor and Outdoor Environment" presented at the Fourth International Symposium on the Natural Radiation Environment, Lisboa, Portugal, December 7-11, 1987

Vanmarcke, H., Reineking, A., Porstendörfer, J., Raes, F. "Comparison of two Experimental Methods to determine the Unattached Fraction of Radon Daughters in Houses" presented at the Fourth International Symposium on the Natural Radiation Environment, Lisboa, Portugal, December 7-11, 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Risø National Laboratory
Health Physics Department
DK-4000 Roskilde

Contract no.: BI6-F-107-DK

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

Dr. J. Roed
Health Physics Dept.
Risø National Laboratory
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 urban surfaces and comparing then to those found in suburban and rural areas. Finding corresponding values of air-exchange rates, indoor deposition parameters, and filtering factors for houses. Finding run-off and weathering parameters for roofs of varying slopes covered with different material.

III. Progress achieved:

The Chernobyl release has provided the project with a substantial amount of useful data.

Dry deposition. More results concerning deposition on urban surfaces have been found from our samples collected in a dry period after the Chernobyl accident; weather conditions were stable with a mean wind speed of 3 m/s and Pasquill stability catagory of B-C. Some of the data are given in Table 1.

Table 1. Deposition on roads

V_d: 10^{-4} ms^{-1}

Isotope	^{134}Cs	^{131}I	^{141}Ce	^{140}La	^{108}Ru	^{95}Zr	^{95}Ne
Urban Areas: asphalt and concrete flagstone	0.7	3.4	7.4	4.8	2.9	6.6	6.5
Suburban: asphalt and concrete flagstone	0.7	5.7	8.5	3.2	4.7	16	9.1
Rural: asphalt	1.1	7.7	11	3.2	2.9	8.2	8.8
Mean total	0.8	4.6	8.2	4.3	3.5	9.9	7.4

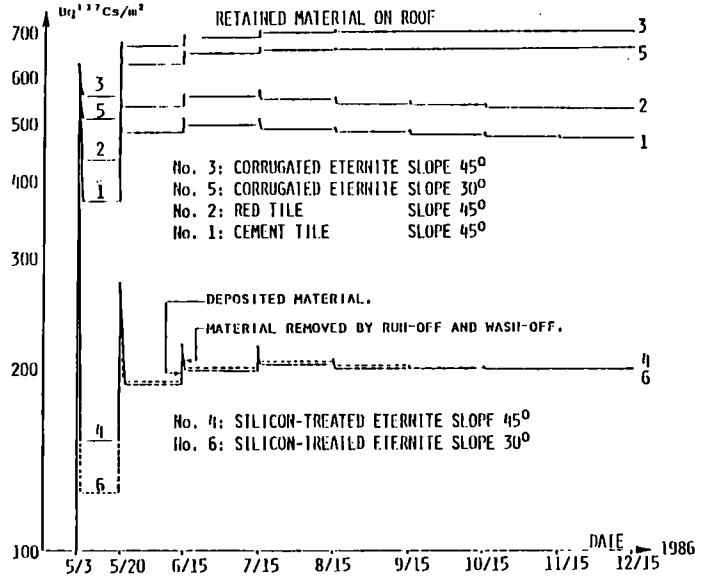
Ventilation, filtering, and internal deposition. The internal rate coefficient of deposition λ_r is found experimentally. λ_r represents the fraction of the aerosols in the air inside a dwelling depositing per unit time. The experiment to find λ_r was designed such that outside air was ducted directly into a test house using a centrifugal blower so that an overpressure was maintained. The air exchange rate, λ_r, and the inlet/-indoor integrated air concentration, C_o/c_i, was measured and λ_r was found as $\lambda_d = \lambda_r\ C_o/C_i^{-1}$). The mean local deposition velocity in the house was then found as $U_d = \lambda_d \cdot V/A$. V is the total volume of the house and A its total internal surface. Some of the the results are shown in Table 2.

Table. 2.

Isotope	U_d, mean deposition velocity: 10^{-4} ms^{-1}	Rate coefficient of deposition λ_d : h^{-1}
^{137}Cs	0.6	0.39
^{134}Cs	0.6	0.38
^{131}J (particulate)	1.1	0.65
^{7}Be	0.7	0.44
^{103}Ru	2.0	1.26
^{106}Ru	1.7	1.02
^{141}Ce	3.1	1.89
^{144}Ce	3.9	2.44
^{95}Zr	5.8	3.56

Run-off and weathering. Run-off and wash-off has been measured during 1986 on six different roof materials, not all of the same slope: cement and red tile with a slope of 45° and corrugated eternite with and without a silicon surface treatment and with slopes of 30° and 45°. The retained ^{137}Cs on the different roofs are shown in Figure 1.

Figure 1.



It is seen that the retention factor for caesium is very high. It has also been shown that the wash-off process is very slow for caesium and Ruthenium. Only a small percentage of the material retained during the first rainfall after the Chernobyl accident was washed off by the end of the year

IV. Objectives for the next reporting period:

Finding dry deposition velocities on pervious urban surfaces especially trees and lawn 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 material in order to quantify them.

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

VI. Publications:

Roed. J. (1987). Dry deposition on smooth and rough urban surfaces. NKA/AKTU-245(87)1. Presented at the Post-Chernobyl Workshop in Brussels 3-5 February 1987.

Roed. J. (1987). Dry deposition in rural and in urban areas in Denmark. Presented at the Workshop on Consequences of an Accidental Contamination of the Urban Environment in Roskilde (Denmark) 9-12 June 1987.

Roed. J. (1987). Run-off from and weathering of roof material following the Chernobyl accident. Presented at the Workshop on Consequences of an Accidental Contamination of the Urban Environment in Roskilde (Denmark) 9-12 June 1987.

Roed. J. and Cannell. R.J. (1987). Relationship between indoor and outdoor aerosol concentration following the Chernobyl accident. Presented at the Workshop on Consequences of an Accidental Contamination of the Urban Environment in Roskilde (Denmark) 9-12 June 1987.

Table. 2.

Isotope	U_d, mean deposition velocity: 10^{-4} ms^{-1}	Rate coefficient of deposition λ_d : h^{-1}
^{137}Cs	0.6	0.39
^{134}Cs	0.6	0.38
^{131}J (particulate)	1.1	0.65
^{7}Be	0.7	0.44
^{103}Ru	2.0	1.26
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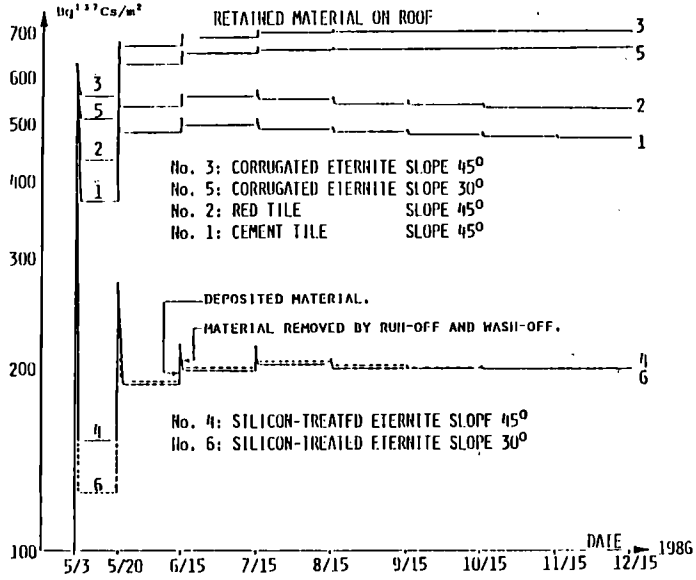
Run-off and weathering. Run-off and wash-off has been measured during 1986 on six different roof materials, not all of the same slope: cement and red tile with a slope of 45° and corrugated eternite with and without a silicon surface treatment and with slopes of 30° and 45°. The retained ^{137}Cs on the different roofs are shown in Figure 1.

Figure 1.



It is seen that the retention factor for caesium is very high. It has also been shown that the wash-off process is very slow for caesium and Ruthenium. Only a small percentage of the material retained during the first rainfall after the Chernobyl accident was washed off by the end of the year.

IV. Objectives for the next reporting period:

Finding dry deposition velocities on pervious urban surfaces especially trees and lawn 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 material in order to quantify them.

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

VI. Publications:

Roed. J. (1987). Dry deposition on smooth and rough urban surfaces. NKA/AKTU-245(87)1. Presented at the Post-Chernobyl Workshop in Brussels 3-5 February 1987.

Roed. J. (1987). Dry deposition in rural and in urban areas in Denmark. Presented at the Workshop on Consequences of an Accidental Contamination of the Urban Environment in Roskilde (Denmark) 9-12 June 1987.

Roed. J. (1987). Run-off from and weathering of roof material following the Chernobyl accident. Presented at the Workshop on Consequences of an Accidental Contamination of the Urban Environment in Roskilde (Denmark) 9-12 June 1987.

Roed. J. and Cannell. R.J. (1987). Relationship between indoor and outdoor aerosol concentration following the Chernobyl accident. Presented at the Workshop on Consequences of an Accidental Contamination of the Urban Environment in Roskilde (Denmark) 9-12 June 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]:

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:

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 dwellings.

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 properties of the exhalation meter and defining a more definite design (optimization);
- exhalation-rate measurements on samples;
- test measurements of the exhalation rate in dwellings and from soil;
- continued study of active-coal canisters and test measurements;
- measurements of radon concentrations (track-etch method) in dwellings, in which a relatively low or high radon concentration was found in the national survey.

III. Progress achieved:

1. Radon exhalation

1.a. Methodology

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 present instrument the radon concentration is determined by collecting radon daughters on a thin foil mounted in front of a ZnS scintillator. The light generated by the alpha's in the ZnS is measured with a photomultiplier-tube.

1.b. Results

The emphasis of the work in 1987 has been on the continued study of the properties of an exhalation meter and trying to define a more definite design. To obtain good counting statistics during the experiments with the prototype instrument a piece of uranium ore was taken as a radon source. As mentioned in last years report, measurements with this instrument showed wiggles in the activity growth curve, which are correlated with changes in light exposure and temperature. It was carefully checked that these wiggles were not instrumental. The wiggles severely influence the deduced exhalation rate: based on our measurements in the laboratory variations of a factor of three should be of no surprise.

Various experiments have been carried out to identify the physics and/or chemistry behind these phenomena. One of the handicaps in such experiments is the time required to measure a growth curve(3-4 days). The experiments indicate that light and temperature changes could affect various parameters and it was decided to design and build an instrument with more controllable parameters. For this purpose the instrument was made of stainless steel to avoid the influence of light exposure and a well defined electric field pulling the charged radon daughters to the foil. Moreover, the instrument is made air tight with possibilities to continuously measure temperature, pressure differences and humidity. Also feed-throughs are present to allow sampling of *radon* by Lucas cells or equivalent devices.

The new device has been placed inside a temperature controlled room. It was found that changes in the equilibrium count rate still occur with changes in temperature. The most surprising result is the fact that the count rate *(radon daughters collected on the foil)* **decreases** with

increasing temperature whilst the *radon* concentration increases slightly. The opposite effect was noticed for a jump to lower temperatures. The *radon* behaviour is consistent with literature where increasing exhalation rates are reported with increasing temperature. Flushing the meter with dry argon gas and repeating the experiment did not change the effects in the count rate.

1.c. Discussion

The results indicate that our present knowledge on the physics inside the instrument is insufficient. We are presently trying to further reduce the number of parameters in order to identify the cause of the ambiguities in the results. We are aware that this physics puzzle delays the realization of the objectives; however, without solving these problems no reliable instrument based on this principle will be achievable. Based on our experiences some doubt has risen on the validity of results obtained by others with similar instruments. Recent comparison, at two other laboratories, of the exhalation rate of the same set of samples by two method revealed results which varied an order of magnitude both ways.

2. Activated charcoal cannisters

Experiments with these cannisters show a strong dependence of the radon-collection efficiency on humidity and temperature. Results of the recent intercomparison meeting at NRPB indicate that futher action has to be taken to obtain reliable results.

3. "High-radon" dwellings

Based on the results of the national radon survey areas with elevated indoor-radon concentrations have been identified. In a number of houses time-averaged radonconcentrations were measured using passive detectors. High radon concentrations were found in the crawl spaces (up to 700 Bq/m^3) in a number of similar houses built in one area. These houses mainly differ in the way the floor characteristics in the crawl space: bare, covered with concrete or concrete covered with a polymer foil. Within the detection accuracy neither a decrease of the concentration in the crawl space, nor in the living room was observed as a result of the covering.

IV Objectives for the next reporting period.

Continued study of the mechanisms causing the large uncertainties in the determination of the exhalation rate;

Construction and test 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.

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

VI Publications:

L.W. Put, R.J. de Meijer and B.F.M. Bosnjakovic, *Radon in the Netherlands*, Proc. APCA Conf "Indoor Radon II", Cherry Hill, N.J.,1987.

F.J. Aldenkamp, L.W. Put and R.J. de Meijer, *Aspects of an instrument for in situ measurements of radon exhalation rates*, Conf. low-level Techniques, Würenlingen, Switzerland, 1987.

F.J. Aldenkamp, L.W. Put and R.J. de Meijer, *Investigations of the properties of an instrument for in-situ exhalation measurements*, Fourth Int. Symp. on the natural radiation environment, Lisboa, Portugal, 1987.

L.W. Put and R.J. de Meijer, *Variations of time-averaged in- and outdoor-radon concentrations with time, location and sampling height*, Fourth Int. Symp. on the natural radiation environment, Lisboa, Portugal, 1987.

L.W. Put and R.J. de Meijer, *Variations of time-averaged outdoor-radon concentrations over a distance of about 50 kilometers,* Fourth Int. Symp. on the natural radiation environment, Lisboa, Portugal, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no.: BI6-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:

- Choice of the type of instrument for continuous radon monitoring suitable for the Dutch situation;
- Assembling a data-acquisition system;
- Select houses for measurements and ask permission from the occupants.

III. Progress achieved:

The search for a full-time group member has been completed succesfully; Mr. P. Stoop could start his activities by August 1st, 1987.

1. Continuous radon monitor.

An inventory study has been made of continuous radon monitors both from descriptions in literature and from information provided by companies selling these instruments. For this purpose contacts with researchers of Lawrence Berkeley Laboratory and Princeton University turned out to be very useful. Discussions with them during visits to houses included in their projects have proven to be of great help in evaluating instruments and measurement techniques. In this evaluation the necessary accuracy in measurements of low radon concentrations turned out to be the limiting boundary condition. Two techniques were selected: a pulsed ionization chamber and a flow-through scintillation chamber. In view of the required volume of 20 l a modular system is preferred. For this reason it was decided to explore the possibilities and limitations of the scintillation chamber first.

Similarly a search has been made for data loggers, able to handle a large set of parameters. After a first selection, bids have been asked from a number of companies. Several parts of equipment to measure related physical parameters have been or are being purchased.

2. Preliminary experiments and calculations

At the end of 1987 a 120 cm tube with a 4 x 4 cm^2 rectangular cross section, coated on the inside with ZnS, has been constructed. At both ends photomultiplier tubes are mounted to detect light pulses. With an α emitting source the efficiency of the light collection is being measured. In this set up the light collection efficiency was measured as function of the distance between source and p.m.-window. The results presented in fig. 1 show that this efficiency decreases exponentially with the distance: a factor of two every 7 cm. This half-value distance may critically depend on the coating procedure of the scintillation material. Our goal, to measure, in time intervals of 30 minutes, concentrations of 5 $Bq.m^{-3}$ with an accuracy of about 20% may be achievable with an improved coating.

Equipment has been purchased to measure, with Lucas cells, high radon

concentrations. The equipment contains a calibration source and may be used for grab sampling as well as continuous measurements. At the intercomparison meeting at NRPB it appeared that the values obtained with this instrument deviated by 15-20% from the refence value; the calibration has been adjusted accordingly.

In the process of evaluating possible measuring methods an attempt has been made to deducte time-dependent *radon-daughter* concentrations from the continuously monitoredsum of *radon and radon-daughter*concentration. These calculations are based on a fourier analysis of the measured *radon* concentrations; they will be further developped.

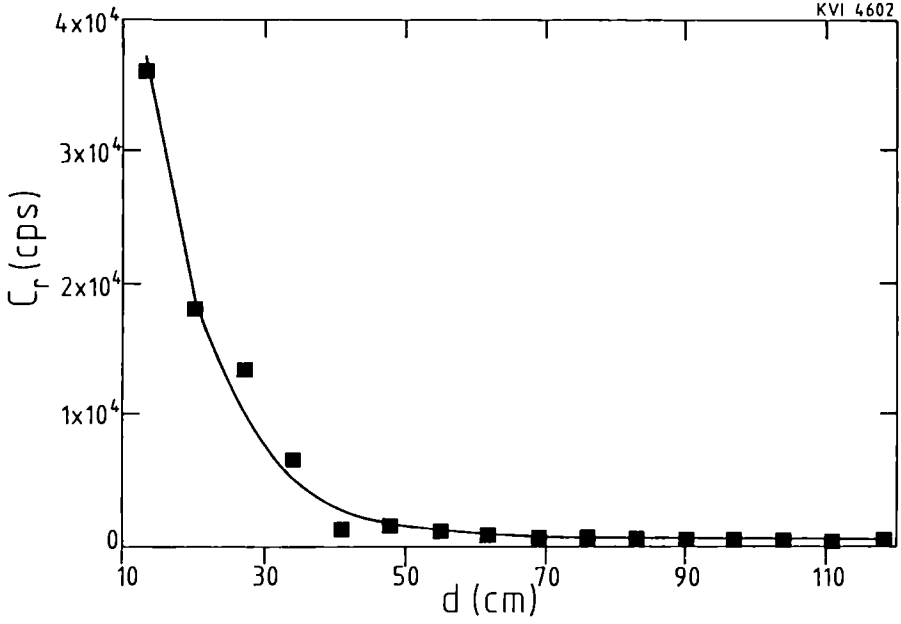


Fig.1. Count rate, C_r, of the photomultiplier tube as function of the distance , d, of an α-source to the tube window. The solid line represents an emperical fit to the data: $C_r = 620 + e^{(11.8-0.1d)}$.

IV. Objectives for the next reporting period:

- Assembling and testing of the continuous radon-concentration monitor;
- installation of equipment in test houses and start of measurements;
- continue with modelling.

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

Prof. Dr. M.B. Greenfield, Florida A&M Universities, Physics Department, Tallahassee Fl 32305, USA.

VI. Publications:

P. Stoop, Instrumenten voor het meten van fluctuaties in de radioactiviteit van lucht; een vergelijkend warenonderzoek, KVI internal report 1145.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor:

Contract no. BI6-F-116-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: (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.

Title of the project no.: 1

Plate-out of radon daughter aerosols in domestic and mine environments.

Head(s) of project:

A C James

Scientific staff:

J C Strong, R A Algar

I. Objectives of the project:

To derive a model that will relate the effective dose equivalent received per unit exposure to radon in air to parameters including the unattached fraction of potential alpha-energy, the equilibrium factor, the aerosol size distribution, aerosol concentration, plate-out velocities and ventilation in both domestic and mine environments.

II. Objectives for the reporting period:

To measure the activity size distribution of the radon daughter aerosol, the unattached fraction of radon daughter potential alpha-energy and the ambient aerosol concentration under representive conditions in dwellings and mines.

III. Progress achieved:

The 5-stage parallel diffusion battery built and calibrated in the previous reporting period was used to study the unattached fraction of potential alpha-energy and the activity size distribution of the radon daughter aerosol in three dwellings and one mine. The concentrations of radon gas and potential alpha-energy were measured simultaneously, with the results given in Tables 1 and 2.

Table 1: Variation of equilibrium factor

Location	No. of measurements	Equilibrium factor, F Mean	Standard deviation
House no. 1 (lounge)	12	0.34	±0.08
(kitchen)	9	0.43	±0.10
House no. 2 (lounge)	3	0.29	±0.01
House no. 3 (bedroom)	9	0.29	±0.10
Gypsum mine	8	0.84	±0.09

The average value of F was about 0.35 for the range of domestic conditions studied, which is lower than the conventional value of 0.5. It is seen from Table 2 that, under domestic conditions, the unattached fraction of potential alpha-energy was generally higher than 0.05, with an average value of about 0.1. The activity median diameter of the attached radon daughter aerosol was typically about 0.13 μm with a geometric standard deviation (gsd) of approximately 2.

Table 2: Characteristics of airborne potential alpha-energy

Location	Unattached fraction (f_p)	Unattached AMD (nm)	attached AMD (nm)	attached gsd
House no. 1				
(lounge)	0.16	< 5	160	1.9
(kitchen	0.16	6	130	1.9
- cooking)	0.10	11	110	1.9
House no. 2				
(lounge)	0.05	6	170	2.0
House no. 3				
(bedroom)	0.15	< 5	130	2.1
Gypsum mine	0.01	2.5	230	2.1

These results and also other recent European studies of aerosol size and attachment in dwellings have been assessed in terms of their effect on the conversion coefficient between exposure to potential alpha-energy and effective dose equivalent using current models of lung dosimetry. It was found that the conversion coefficient decreases from about 50 to 10 nSv per Bq (EERn) h m^{-3} as the equilibrium factor increases from about 0.2 to 0.8. However, the dose was found to be more closely represented by the time integral of radon gas concentration, with the results shown in the figure. A reference conversion coefficient of 1 mSv y^{-1} per 20 Bq m^{-3} average radon concentration was derived for domestic conditions (James et al., in press).

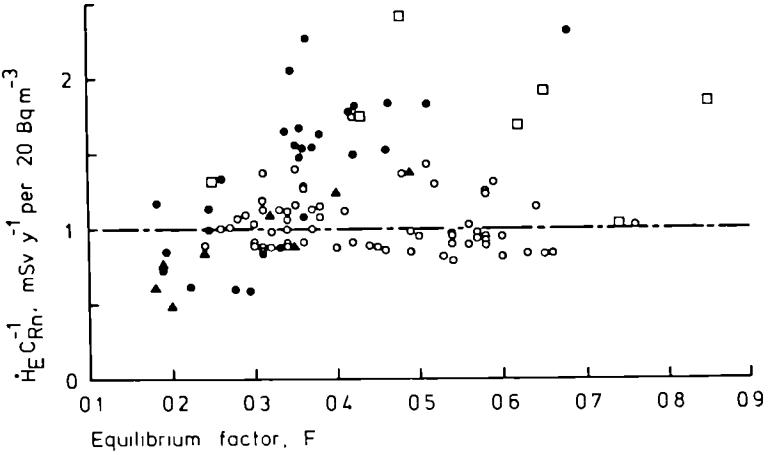


Figure: Values of annual effective dose equivalent for exposure to an average concentration of radon gas of 20 Bq m^{-3}. Results from this study are shown as solid circles. The additional symbols represent data from other European studies.

IV. Objectives for the next reporting period:

To complete measurements for a representative range of dwellings and domestic conditions and for mines differing in ventilation and working characteristics. To assess the validity of radon exposure as a general index of dose. To identify any modifying parameters, conditions under which they may need to be applied, and the relative doses per unit exposure between mines and dwellings.

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

None in this period.

VI. Publications:

Strong J C and Tu K W. Design and calibration of a multichannel diffusion battery for the measurement of radon daughter activity-size distributions. IN: Aerosols, their behaviour and applications. Proc. of 1st Conference 31 Mar - 1 Apr, 1987, Loughborough. The Aerosol Society, Loughborough, pp 21-24 (1987).

James A C. A reconsideration of cells at risk and other key factors in radon daughter dosimetry. IN: Radon and Its Decay Products: Occurrence, Properties and Health Effects. P K Hopke, ed. American Chemical Society, Washington, DC, 400-418 (1987).

James A C, Strong J C, Cliff K D and Stranden E. The significance of equilibrium and attachment in radon daughter dosimetry. IN: Proc. 4th International Symposium on the Natural Radiation Environment, Lisboa, Portugal, December 7-11 1987.

Title of the project no.: 2

Deposition of aerosols in the upper respiratory tract.

Head(s) of project:

A C James

Scientific staff:

J C Strong, R A Algar

I. Objectives of the project:

To measure the filtration efficiency of the human nose for sub-micron particles in the size range of the natural radon daughter aerosol and to establish a model to predict deposition as a function of particle size and flow rate. To determine the effect of airway dimensions on deposition and its dependence on age. To determine the sites and magnitudes of deposition within the nasal passages in order to assess doses to epithelial tissues.

II. Objectives for the reporting period:

To extend the range of the monodisperse particle generator to produce particles down to 5 nm diameter and complete studies of penetration through a hollow cast of the human nose as a function of particle size and flow rate. To measure nasal penetration of unattached polonium-218 as a function of flow rate. To investigate the distribution of deposited thoron daughters within a nasal cast.

III. Progress achieved:

The output and stability of the condensation aerosol generator was increased substantially for very small particles down to 5 nm diameter by cooling rapidly particles of silver flushed from a furnace with a flow of nitrogen gas. The penetration of monodisperse particles through a hollow cast of the human nose, measured at various flow rates using the apparatus described in the previous report, is shown in Figure 1 (Strong and Swift, 1987).

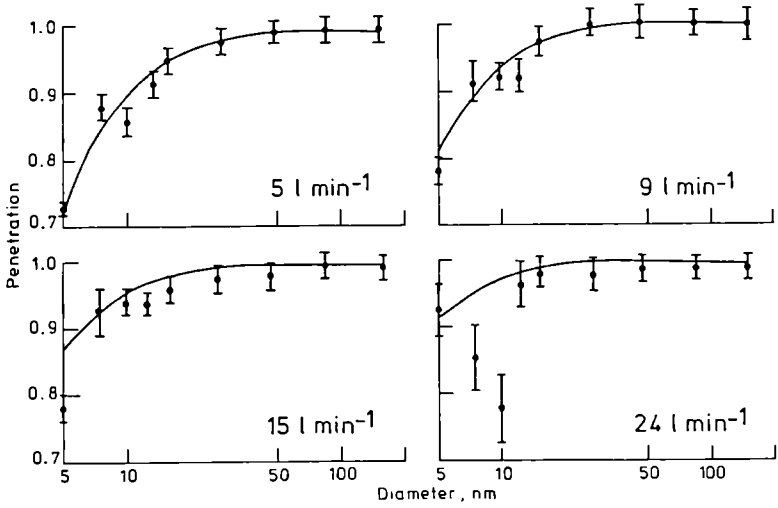


Figure 1: Penetration through a nasal cast of aerosol particles in the size-range 5 - 150 nm diamter at four flow-rates, with curves calculated for a cylindrical tube 750 mm long.

The experimental apparatus built to measure the penetration of unattached Po-218 through a nasal cast is shown in Figure 2. The Ra-226 source (Pylon, Canada) served as a closed circuit generator of radon gas. After filtration to remove airborne particles, the circulating air entered a growth tube where unattached Po-218 was formed. The output was split equally between the nasal cast and a filter using identical pipework to ensure equal desposition loss. The Po-218 atoms that penetrated the cast were sampled by an identical filter. The fractional penetration through the cast was calculated from the ratio of activities collected on the two filters after correcting for deposition loss in the pipe leaving the cast. The airborne concentration of condensation nuclei in the circuit was monitored and shown to be less than 20 cm^{-3}. The proportion of Po-218 activity attached to particles was therefore negligible.

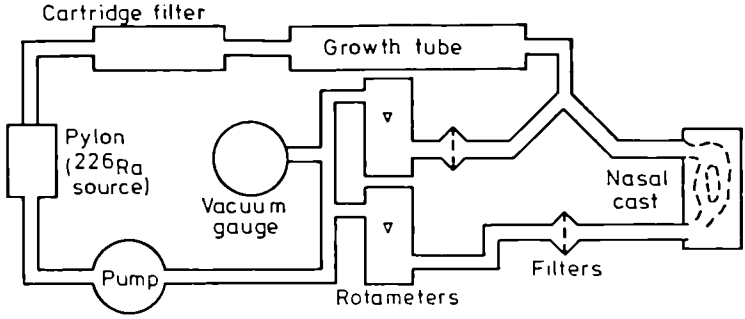


Figure 2: Schematic diagram of the apparatus used to measure penetration of unattached Po-218 through the nasal cast.

The average fractional penetrations measured at flow rates of 5, 11.5 and 18 litres per minute were 0.4, 0.46 and 0.51, respectively. According to the deposition tube model shown in Figure 1 to represent penetration as a function of particle size, the equivalent diffusion diameter of unattached Po-218 was found in these tests to be approximately 2 nm. It can be concluded that the fraction of unattached radon daughters that deposits in the human nose is approximately 0.5, with relatively little dependence on flow rate over the normal respiratory range. Likewise, it can be concluded that radon daughter activity carried by particles larger than about 10 nm diameter penetrates the nasal passages without loss. These experimental data are shown in Figure 3 to be consistent with the single value of nasal deposition measured for 5 nm particles in human subjects by Schiller (1985).

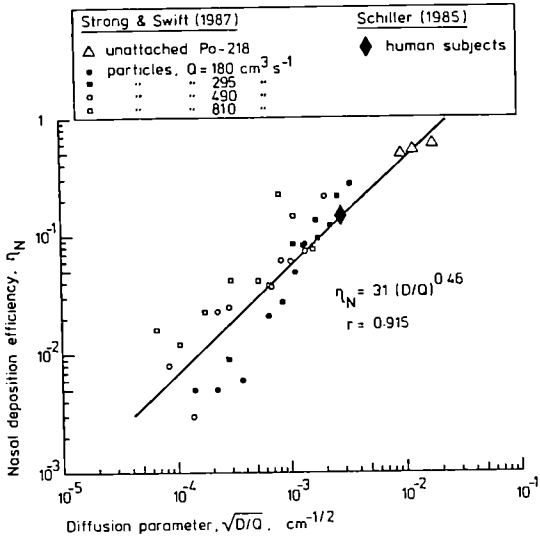


Figure 3. Deposition efficiency for submicron particles measured at various flow rates in a hollow cast of the human nose plotted as a function of the diffusion parameter i.e. the square root of the quotient of particle diffusion coefficient and flow rate. The single value measured in human subjects is shown for comparison.

The experimental apparatus shown in Figure 2 was used with a Th-228 source to expose the nasal cast to unattached Pb-212 atoms. Exposures of several hours duration were carried out at three different flow rates to give about 150 kBq of deposited activity in each case. The distribution of Pb-212 activity within the exposed cast was measured using a gamma camera at the Department of Nuclear Medicine, Radcliffe Infirmary, Oxford (Dr N Soper). The gamma camera image was divided into two fields of interest, corresponding to the anterior and posterior regions of the nose. These correspond to sites of deposition where material clears forwards to the nostrils and backwards to the pharynx, as described by Swift and Proctor (in press). The proportion of activity deposited in the anterior region was found to have a constant value of about 65% for the three flow rates, 5, 12 and 19 l min^{-1}.

References

Schiller, Ch.J. Diffusionsabscheidung von Aerosolteilchen im Atomtrakt des Menschen. PhD Thesis, J W Goethe-Universitat, Frankfurt/Main (1985).

Swift D L and Proctor D F. A dosimetric model for particles in the respiratory tract above the trachea. Ann. Occup. Hyg. (in press).

IV. Objectives for the next reporting period:

To measure the effect on nasal penetration of smaller airway size in children, using hollow casts prepared by Dr Swift. To evaluate doses to nasal epithelial tissues from localised deposition observed in the hollow cast.

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

Dr D L Swift, Associate Professor, Dept. of Environmental Medicine, Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205, U.S.A.

VI. Publications:

Stong J C and Swift D L. Deposition of ultrafine particles in a human nasal cast. IN: Aerosols, their generation, behaviour and applications. Proc. of 1st Conference 31 March - 1 April, 1987, Loughborough. The Aerosol Society, Loughborough, pp 109-112 (1987).

Title of the project no : 3

Application of CR-39 track-etch detectors to low background counting and particle sizing of air samples.

Head(s) of project:

A C James

Scientific staff:

J C H Miles, A Birchall, 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 sample filters.

II Objectives for the reporting period:

To develop and test software for automatic scanning and assessment of etched-track autoradiographs.

III. Progress achieved:

A contract has been let with the Medical Research Council, Population Cytogenics Unit, Edinburgh, to develop software as specified by NRPB to enable the Board Cytoscan 110 optical image analyser to read alpha tracks in chemically etched CR-39 detectors. The software is required to identify automatically and record the position of each track together with other parameters such as projected area, grey level and the lengths of the perimeter and major and minor axes. Two versions of the software have been written and tested. The first was found to operate unreliably on the NRPB Cytoscan hardware. Most of the operating faults were corrected in the second version, which has been tested with autoradiographs of radon daughters collected on personal air sampler filters. The autoradiographs were etched for various lengths of time and also had a range of track densities. These tests demonstrated that the software can identify tracks correctly from their shape, even when they vary widely in size, and can record the required parameters of accepted tracks. Spurious images are rejected reliably.

Comparison of automatic and manual counting has demonstrated that the Cytoscan result is generally a constant proportion of that by eye. In exceptional cases, a disproportionately low Cytoscan result was due to failure of the automatic focussing or to high track density when the system memory overflows at too high a rate of data acquisition. These faults will be corrected by optimising the mechanical focus slew and scanning rates and, if necessary, by modifying the software control routine.

Tests with autoradiographs of radon daughters and plutonium particles have identified other areas where the Cytoscan software must be improved. In particular, the optical magnification needs to be optimised for tracks of widely different size, and the mechanical scanning rate needs to be varied automatically with track density. The latter is necessary to avoid memory overflow and loss of data when tracks occur in dense localised clusters and also diffuse in the same sample. Individual clusters must be scanned relatively slowly, whereas the diffuse track pattern should be scanned rapidly in order to minimise the overall counting time. A third version of the software which is addressed to these points is being developed.

IV. Objectives for the next reporting period:

Final testing will be undertaken of Cytoscan software with a wide range of autoradiographs, especially for non-uniform track densities given by plutonium particles collected on personal air sample filters. Development of mathematical techniques and software to assess sampled activity and particle size distribution from the recorded track count and spatial pattern.

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

Population Cytogenics Unit, Medical Research Council, Edinburgh. (Dr. D Rutovitz).

VI. Publications:

The probability of plutonium intakes and doses exceeding estimates from personal air sampling. A Birchall, A C James and C R Muirhead, 1988. NRPB R-212.

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 publish papers describing (i) a methodology for distinguishing relative and absolute risk models for radiation-induced cancers, and (ii) its application to data on the Japanese atomic bomb survivors and U.K. patients with ankylosing spondylitis given x-ray therapy.

III. Progress achieved.

1. Methodology

Two possible representations for the cancer rates in an irradiated population relative to those in an unirradiated population are (i) a relative risk model, under which these rates differ by a factor, and (ii) an absolute risk model, under which the rates differ by an absolute value. Here the factor or absolute value may depend on terms such as sex, age at exposure and possibly time since exposure. Allowance can also be made for a minimal latent period. For any particular set of factors, the fit of the relative and absolute risk models can be compared on the basis of a generalised risk model that simplifies to either the relative or the absolute model as a special case. The fit of the generalised model to the data can then be compared directly with that of each of the relative and absolute models. In addition, the fit of the generalised models for different nested sets of terms can be compared.

These methods have been published in a paper presented to the Royal Statistical Society in January 1987 (see VI, 1). They have been applied to data from the Hiroshima atomic bomb survivors (followed to the end of 1978) and the UK ankylosing spondylitis patients given x-ray therapy (followed to the beginning of 1970). Results were published both in the above paper and in a paper presented at a conference organised by the British Nuclear Energy Society in London, May 1987 (see VI, 2).

2. Results

For mortality from tumours of epithelial cells for non-sex-specific sites and for breast cancer incidence (among the atomic bomb survivors), a generalised risk model dependent on at most sex and age at exposure (but not time since exposure) provides a good fit to the data. While the relative risk model dependent on these terms is consistent with the generalised model, the corresponding absolute risk model is not. Thus a model under which the relative risk is constant with time since exposure (following a minimal latent period) is to be preferred to the corresponding model based on absolute risk.

The same conclusion holds for the grouping of all cancers other than leukaemia among the Hiroshima atomic bomb survivors. However, these data are also consistent with models under which the relative or absolute risk additionally depend on time since exposure. In particular, depending on whether the radiation-associated cancer risk is modelled off as tailing off after the period of follow-up (ie. 33 years after exposure in the case of these data) or as continuing to increase with time, the predicted lifetime cancer risk can vary by an order of magnitude.

For leukaemia mortality, differing results were obtained for the spondylitis patients and for the Hiroshima atomic bomb survivors. In the former case a model under which the relative risk varies solely with time since exposure provides a good fit to the data, whereas the corresponding absolute risk model does not fit well. However, the reverse is true for the latter population.

3. Discussion

For the disease groupings of solid cancers and all cancers other than leukaemia described above, the data studied are consistent with a model under which the relative risk is constant with time since exposure (following a minimal latent period), but not with the corresponding absolute risk model. However, the data are also consistent with more complex risk projection models that produce widely differing predictions of lifetime risk. Hence data from longer follow-ups of populations exposed to high doses are required.

In the case of the Japanese atomic bomb survivors such data have just become available following the publication by the Radiation Effects Research Foundation (RERF) of Life Span Study Report 11, covering the period up to the end of 1985. It is intended to obtain these data from RERF in order to study not only the topic of risk projection, but also the effect of the revised dosimetry for the Japanese survivors. Follow-up of the ankylosing spondylitis patients to the end of 1982 has been analysed in a paper by Darby et al (British Journal of Cancer, 55, 179-190, 1987) from the Imperial Cancer Research Fund; here the radiation-related cancer risk seemed to be tailing off by 25 years after exposure. The above methods may be applied to these data at a later date. Data from the Colorado uranium miners may also be studied.

IV. Objectives for the next reporting period:

To obtain data from the latest follow-up of the Japanese atomic bomb survivors, and to analyse the effect of the revised dosimetry and the choice of risk projection model.

To analyse any other data sets on populations exposed to high radiation doses that may become available; for example, for the Colorado uranium miners.

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:

(1) Muirhead, C.R. and Darby, S.C., Modelling the relative and absolute risks of radiation-induced cancers (with discussion). Journal of the Royal Statistical Society, A150, 83-118 (1987).

(2) Muirhead, C.R. and Darby, S.C., Distinguishing relative and absolute risk models for radiation-induced cancers. In Proceedings of the BNES Conference on Health Effects of Low Dose Ionising Radiation - Recent Advances and their Implications, London, May 1987 (to appear).

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 epdemiological 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 publish further details of the computer program written to analyse occupational data; to continue the refinement and validation of this program.

To publish a review of methods for monitoring the health of communities living near nuclear installations. To examine statistical methods for analysing data on such populations.

III. Progress achieved

1. Methodology

The computer program ARFAR (At Risk For Any Reason) has been written at NRPB to assist in the analysis of epidemiological data for those exposed occupationally to ionising radiation. The program allows person-years-at-risk to be stratified by cumulative radiation dose, as well as by standard variables such as age, calendar period, etc. On the basis of the output from the program, the internal test for a trend in cancer rates with dose that was described by Darby and Reissland (Journal of the Royal Statistical Society, A144, 298-331 (1981)) can be performed. Details of the program were given at a Royal Statistical Society Conference in April 1987 (see VI, 2) so that refinements could be made in the light of any comments from scientific colleagues.

On the topic of epidemiological studies of the public, technical considerations underlying the use of routinely-collected small area statistics have been studied (see VI, 1). Methods for assessing whether a particular disease, such as leukaemia, has a natural tendency to "cluster" on a geographical basis have also been examined. Various such methods have been used to form a backdrop in published studies of cancer around nuclear installations. An attempt has been made to determine the optimal method based on data for areas with unequal population sizes.

2. Results

Following a suggestion made at the above conference, work has been carried out on modifying ARFAR so that more general latency distributions for risk following exposure can be accommodated. In particular, rather than assuming that any cancer risk suddenly commences at a specific time following exposure (ie. in the form of a step function), ARFAR is being modified so that the risk can alternatively increase linearly with time (in the form of a ramp) until it reaches a plateau. Work is continuing on this topic.

A program for simulating occupational mortality data based on personal dose-distories from the Hanford (USA) work-force, namely SIRIS (developed by GSF, Neuherberg - see V), has been installed at NRPB. The aim is to use this program as part of the procedure for validating the output from ARFAR. However, owing to differences between the type of FORTRAN code in which SIRIS was written and that which can be used in the UK, it has not as yet been possible to run this program at NRPB.

As regards epidemiology of the general population, there are difficulties in England and Wales in obtaining consistently defined geographical areas to allow monitoring of disease trends. The techniques used in recent epidemiological studies have been reviewed (see VI, 1). Here "cusum" techniques may help in detecting temporal changes in disease rates. On the topic of tests for "natural clustering", a search of the literature indicated that a test derived by Potthoff and Whittinghill (Biometrika, 53, 167-182 and 183-190) should be optimal at detecting "over dispersion" relative to the Poisson distribution in the numbers of cases of a disease. This test does not appear to have been used in an epidemiological setting up till now; details of it have been passed on to interested epidemiologists.

3. Discussion

Further work is required on refining and validating ARFAR. In particular, providing it is possible to eventually run SIRIS at NRPB, tests on simulated data will be undertaken. Following this it is intended to use ARFAR in the analysis of data from the U.K. National Registry for Radiation Workers (funded partially under CEC contract BI6-F-213-UK). Copies of the program will continue to be made available to other research workers.

With regard to tests for clustering of disease in the general population, study is required of how tests such as that described above would perform in practice; a Monte Carlo approach based on actual population sizes may help in this. For example, the statistical power of the above test is likely to depend on the size of the geographical area over which any natural clustering tends to take place. Thus analysis may be made of how such a test can be modified so that it distinguishes "short-range" and "long-range" clustering effects.

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 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:

Publication in Scientific Journal

(1) Ennis, J.R., How should the health of communities near nuclear installations be monitored? Journal of the Society for Occupational Medicine, 37, 19-23 (1987).

Presentation at Scientific Conference

(2) Ennis, J.R., Ball, A.M. and Barry, S.F., ARFAR - A person-years-at-risk program for radiation dosimetry data. Presented at the Royal Statistical Society Charter Centenary Conference, Cambridge, 8-10 April 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB- Oxon OX11 ORQ

Contract no. B16-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

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.

The database for the National Registry for Radiation Workers (NRRW) continues to extend and improve in quality. One longstanding problem has been the relatively large number of individuals who could not be flagged with the National Health Service Central Register (NHSCR). This is necessary if the study is to be notified of details of deaths. At the time of the first supplement to the protocol[1] 61% of participants had been flagged with the NHSCR; in the second supplement[2] this has risen to 74%. The third supplement[3] will be published shortly and will show that 88% of individuals have been flagged. More work remains, however, before the target of 95% flagging is reached. Attention is also being given to other missing data.

The NRRW is somewhat unusual in that individuals are given the opportunity to refuse to participate. On the advice of the Advisory Committee for the NRRW, participating organisations have been asked to provide anonymous statistical data for non-responders so as to check that they do not differ systematically from participants. An example of these data are given in the table, where dose distributions for participants and non-participants is compared. It can be seen that the distributions for the two groups are broadly similar. The table also gives the collective doses incurred by individuals in different dose categories. They are discussed further below.

Plans are also in hand to undertake the first analysis of the NRRW. This will involve completion of the database (obtaining all necessary data up to the follow-up date), data validation and then the analysis itself. This is described in a document to be published shortly[4]. It will consist of a comparison with national mortality rates and tests for trends of mortality rates with dose. The NRRW does not have a control population of un-exposed individuals and it is likely that the healthy worker effect will be observed so that mortality rates will be lower than in the general population. However, as the table shows, the bulk of individuals have received relatively little radiation and comparisons between individuals who have received different radiation doses will be the most revealing part of the analysis.

Table

	Dose range (lifetime dose) <10 mSv	10–50 mSv	>50 mSv	Total
No. of employments: Participants	52347 (55%)	26529 (28%)	15969 (17%)	94845 (100%)
Non-participants	56%	30%	14%	100%
Collective dose for participants (Sv)	133 (4%)	577 (19%)	2266 (76%)	2976 (100%)

References

(1) Darby, S.C. and Saw, G.M.A., Summary of data held by the National Registry for Radiation Workers : Supplement to the Protocol. Chilton: NRPB-R116 (London: HMSO) (1982).

(2) Saw, G.M.A. and Kendall, G.M., Second Supplement to the Protocol. Chilton: NRPB-R116 (1985).

(3) Kendall, G.M., O'Hagan, J.A., Rees, S., Walker, S.M. and Muirhead, C.R., Third Supplement to the Protocol. Chilton: NRPB-R116 (1988).

(4) Ennis, J.R., Kendall, G.M. and Muirhead, C.R., Proposals for analysis of the data held by the National Registry for Radiation Workers. To be published.

IV. Objectives for the next reporting period:

To publish both the third supplement to the Registry Protocol and proposals for analysis of the data.

To attempt to complete the data base up to the follow-up date (intended to be end of 1985) and undertake data validation.

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:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]

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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 cost, 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 IRRADIATI9NS INDUSTRIELLES/BASE DE DONNEES EUROPEENNES

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 des rejets de l'industrie nucléaire pour les populations des pays de la CEE (évaluations faisant appel, d'autre part, à des modèles de dispersion à longue distance : modèles transfrontières).

II. Objectives for the reporting period:

- Mise à disposition sous forme de fichiers informatiques des résultats de l'exploitation des données agricoles détaillées de l'Angleterre et du Pays de Galles (mailles de 100 km²).
- Répartition de la population de la Grèce et de l'Espagne (traitement des données acquises en 1986).
- Répartition des classes d'utilisation du territoire de la CEE, en larges mailles (10 000 km²) à partir des données régionales.

(tant dans le cadre du projet MARIA que dans le contexte de Tchernobyl).

III. Progress achieved:

- Les résultats du traitement des données du recensement communal de 1981 concernant les surfaces agricoles et les effectifs du bétail en Angleterre et au Pays de Galles (données fournies au niveau du km² dans la grille britannique) ont été regroupés sur fichiers informatiques, par classes de produits et par mailles de 100 km² de la grille européenne. Ils ont servi au calcul de la répartition des productions végétales et animales importantes du point de vue des études de protection, au moyen de coefficients de rendement appropriés (moyennes nationales ou régionales selon les cas). Les quantités livrées à la consommation, estimées à l'aide de coefficients choisis en accord avec le N.R.P.B., sont aussi réparties dans la grille selon leur lieu d'origine. Des tableaux détaillés ont été édités aux fins d'analyse et de vérification. Cet examen (qui fait l'objet d'un rapport interne) montre que les transformations opérées pour permettre l'application des modèles de calcul de doses aux populations n'altèrent pas la validité des résultats.

- Les populations de la Grèce et de l'Espagne ont été réparties dans les mailles européennes de 10 000 km² :

  . la première (Grèce) par exploitation manuelle des données de recensement fournies au niveau des provinces (au nombre de 52), à l'aide de cartes détaillées. Le résultat est approximatif en raison de l'hétérogénéité du territoire ;

  . la seconde (Espagne) par traitement automatique des données communales accompagnées des coordonnées géographiques. Ceci a permis d'obtenir en même temps la répartition dans la grille des surfaces régionales, nécessaire à l'exploitation de diverses données statistiques. La population des villes de plus de 19 000 habitants a été mise en évidence, ainsi que la répartition des communes en fonction de leur altitude ;

  . les données régionales dites d'"Utilisation du territoire" issues de l'Office Statistique des C.E. et entrant dans la base de données "REGIO" sont présentées dans le réseau de mailles de 10 000 km². Pour cela, les diverses utilisations ont été réparties proportionnellement aux surfaces des diverses régions contenues dans chaque maille ce qui suppose l'uniformité à l'intérieur de chaque région : hypothèse évidemment grossière, mais valable en première approximation vu l'étendue du territoire de la Communauté ;

  . enfin, on a montré la possibilité d'associer des données physiques présentées sous la forme circulaire classique (roses de concentrations ou de dépôts radioactifs) aux données démographiques, agricoles ou socio-économiques du réseau, grâce à un programme de changement de configuration.

IV. Objectives for the next reporting period:

- Répartition d'autres données socio-économiques (en particulier des productions agricoles) des pays de la C.E. par mailles de 10 000 km² à partir des données régionales disponibles, à l'aide des coefficients appropriés.

- Composition d'une notice rassemblant définitions, nomenclatures et programmes de transformation en vue, soit de l'harmonisation et de l'introduction des données, soit de l'utilisation des résultats.

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

VI. Publications:

A. GARNIER - General data base in european grid model (MARIA contractors meeting, Brussels, 2nd Feb. 1987)

A. GARNIER - La grille européenne : un cadre géographique d'actualité pour l'harmonisation des études relatives à la radioactivité de l'environnement et à son impact sanitaire (Congrès Franco-Italien, Castelgandolfo, 12-13 Oct. 1987)

A. GARNIER - Progress in the preparation of demographic and land use data bases (to be presented at MARIA Workshop, Roma, Janv. 1988).

Title of the project N° 2 :

EVALUATION DU DETRIMENT OBJECTIF GLOBAL ET DE SON COUT EN RELATION AVEC LES CONSIDERATIONS ECONOMIQUES - RECHERCHES :
a. sur l'homme (R. MAXIMILIEN)
b. Sur l'animal (M. DALEBROUX)

Head(s) of project : a. R. MAXIMILIEN
b. M. DALEBROUX

Scientific staff : a. R. MAXIMILIEN
b. M. DALEBROUX

I. Objectives of the project :

a. Sur l'homme :

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.

b. Sur l'animal :

Fournir une aide à l'interprétation statistique de données recueillies par le laboratoire de Radiopathologie et de Toxicologie Expérimentales du Commissariat à l'Energie Atomique à Fontenay-aux-Roses.

II. Objectives for the reporting period :

a. Sur l'homme :

1. Rédaction d'un rapport de synthèse sur les méthodes d'évaluation des cancérigènes chimiques faisant le point des possibilités et des limites d'une intercomparaison avec les rayonnements ionisants.

2. Illustration des conclusions sur l'exemple du nickel qui représente un des cancérigènes chimiques les mieux documentés sur le plan sanitaire.

b. Sur l'animal :

1. Procéder à l'analyse statistique d'un ensemble de données et proposer des tests de signification sur la mesure de l'Efficacité Biologique Relative de deux expositions, à des rayonnements gamma d'une part et à des neutrons d'autre part. Cette première analyse, portant sur les cancers totaux, a déjà fait l'objet d'un compte-rendu dans le Progress Report de 1986.

2. Affiner l'analyse ci-dessus en distinguant les carcinomes des sarcomes, chacune des deux catégories étant elle-même partagée en deux classes.

3. Enfin, prendre en considération les seuls cancers pulmonaires pour des expositions à des rayonnements gamma, des neutrons et du Radon.
N.B. - Cf. point III pour les détails.

III. Progress achieved :

a. Sur l'homme :

Principales conclusions des études documentaires :

Les points de comparaison des évaluations humaines du risque dans chacun des domaines apparaissent peu consistants (spectres des affections malignes induites, susceptibilité des organes, etc..).

La compréhension des mécanismes de la cancérogénèse représente le champ de confrontation le plus intéressant ; la complexité du processus nécessite le recours à des indicateurs dont la pertinence diffère significativement selon les cas : (i) évaluation de l'atteinte de la cible avec les réactions métaboliques d'activation et de détoxification propres aux agents chimiques (ii) évaluation des conséquences des interactions biologiques avec des indicateurs de dommage témoignant de l'atteinte du matériel génétique (seuils métaboliques dans le cas du chimique +++) et des indicateurs d'effet corrélés plus ou moins étroitement au processus et pouvant prendre, fait remarquable, une signification différente selon les classes d'agents (relations dose-effets clastogènes-cancérogénèse établies ou non en fonction des divers agents).

Pour ce qui concerne le cas du nickel, un inventaire critique des données comptables issues de l'épidémiologie, de l'expérimentation animale et des divers tests in vitro a été dressé pour quelques composés en vue de procéder à la mise en oeuvre ultérieure d'une approche standardisée d'intercomparaison des potentiels cancérigènes.

b. Sur l'animal :

1. On a analysé des données fournies par le laboratoire de Radiopathologie et de Toxicologie Expérimentales du C.E.A. Ces données consistaient en nombres de cancers par rat et l'évolution de ces nombres en fonction de l'âge des animaux, compte tenu des doses absorbées en début d'expérience soit en débit faible, soit en débit fort.

Toutes les réponses étaient de type exponentiel positif ; elles ont été linéarisées pour les analyses de variances, lesquelles ont permis de repérer deux groupes de réponses différents, chacun caractérisé par des droites considérées comme statistiquement parallèles. Les techniques de calcul à la base de ce genre d'analyse, mettant en oeuvre des tests de parallélisme, sont classiques ; elles ne font donc pas l'objet d'un développement particulier dans ce rapport.

On a ensuite proposé des tests de signification sur la mesure de l'Efficacité Biologique Relative (EBR) de deux expositions sur la base des réponses obtenues par l'analyse statistique. A ce propos, on a distingué deux cas différents faisant appel, bien entendu, à des tests distincts :

- EBR sur deux réponses du même groupe, donc sur des droites parallèles ;

- EBR sur deux réponses appartenant à des groupes différents, donc sur des droites quelconques.

2. Pour une dose déterminée, aussi bien pour le rayonnement gamma que pour les neutrons et les témoins, la vitesse d'apparition, en fonction de l'âge, des carcinomes était systématiquement plus grande que celle des sarcomes. Ceci restait vérifié lorsque la comparaison était faite après le partage en deux classes de chacune des deux catégories de cancers.

3. En ce qui concerne les cancers pulmonaires observés pour les plages de doses utilisées, la vitesse d'apparition la plus élevée a été constatée pour le rayonnement gamma, et la plus faible pour les neutrons. Pour le Radon, on a obtenu une vitesse intermédiaire.

IV. Objectives for the next reporting period :

a. Sur l'homme :

Procéder à l'analyse comparative des effets génotoxiques et mutagènes entre métaux non ferreux et rayonnements ionisants (étude documentaire).

b. Sur l'animal :

Continuation de la collaboration avec le laboratoire de Radiopathologie et de Toxicologie Expérimentales du C.E.A. à Fontenay-aux-Roses.

V. Publications :

a. Sur l'homme :

F. DUFOUR, R. MAXIMILIEN, C. CARGOU - Réflexions sur une approche méthodologique pour l'analyse de la littérature scientifique à propos des métaux et de leurs composés
3ème Journée Scientifique de Suze-la-Rousse, Hygiène Industrielle, Suze-la-Rousse, 16-18 Septembre 1987.

R. MAXIMILIEN - Comparaison du détriment radiologique et chimique chez l'homme -
Revue de la littérature (à paraître).

Title of the project no.: 2

EVALUATION DU DETRIMENT OBJECTIF GLOBAL ET DE SON COUT EN RELATION AVEC LES CONSIDERATIONS ECONOMIQUES -
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:

Etudes générales post-Tchernobyl.

II. Objectives for the reporting period:

1. Comparer les différents modèles utilisés pour évaluer les conséquences de l'accident de Tchernobyl ; comparer les résultats de ces modèles aux mesures.

2. Etablir une synthèse des mesures anthropogammamétriques effectuées dans les différents pays, suite à l'accident de Tchernobyl.

III. Progress achieved:

1. Comparaison des différents modèles et des mesures

a/ Les différentes séquences d'émission, prises une à une, conduisent à des évaluation de concentrations dans l'air, à grande distance du point d'émission, qui peuvent être très différentes selon les modèles utilisés. Toutefois, si l'on considère l'ensemble de la période du rejet (une quinzaine de jours), il ne semble pas qu'il y ait un modèle qui donne des résulgats significativement et systématiquement meilleurs que les autres.

b/ Cette étude a également mis en évidence la difficulté, voire l'impossibilité de comparer des résultats de mesures effectuées par différents laboratoires : les différentes techniques de prélèvement, de mesure et de présentation des résultats rendent délicat ce type de comparaison, et la recherche d'une harmonisation des procédés et des méthodes conduisant du prélèvement au résultat, en passant par la préparation de l'échantillon et sa mesure, est un préalable nécessaire à de telles entercomparaisons.

2. Synthèse bibliographique des mesures anthropogammamétriques

L'ensemble des mesures anthropogammamétriques effectuées en Europe montrent que les expositions les plus importantes ont été observées en Bavière, dans le Tessin et, dans une moindre mesure, en Italie du Nord. Il semble que les évaluations d'incorporation effectuées à partir des mesures de dépôt et de concentration atmosphérique soient systématiquement supérieures aux mesures anthropogammamétriques (jusqu'à un facteur 10).

IV. Objectives for the next reporting period:

Participer aux éventuelles actions communautaires visant à harmoniser la mesure des radionucléides dans les différents compartiments de l'environnement.

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

VI. Publications:

A. DESPRES - The Chernobyl accident : comparison of models used and of impacts of national or regional countermeasures - Workshop on the assessment of the radiological consequences of the Chernobyl accident - Bruxelles, 3-5 Février 1987

A. DESPRES - L'accident de Tchernobyl - Problèmes liés à la modélisation et à la métrologie - Congrès Franco-Italien AIRP/SFRP - Csstelgandolfo, 12-13 Octobre 1987

A. DESPRES - Les mesures anthropogammamétriques effectuées en Europe après l'accident de Tchernobyl - Synthèse bibliographique - IPSN-DPS/SEGP, Note LSEES 87-18

TITRE DU PROJET n° : 3

Evaluation des dimensions subjectives du detriment radiologique en relation avec les considérations sociologiques.

CHEF DE PROJET : J.BRENOT

EQUIPE SCIENTIFIQUE : J.BRENOT, S.BASTIDE

I. OBJECTIFS DU PROJET

L'analyse des attitudes du public envers l'énergie nucléaire montre l'importance des composantes subjectives dans l'appréciation du risque. L'expérience, la peur, 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, ceci conduisant en quelque sorte à l'objectiver.

II. TACHES DE L'ANNEE 1987

1. Etude bibliographique.
2. Résultats de la seconde enquête sur les situations à risque chez les travailleurs du nucléaire (Centre de Saclay).
3. Résultats de l'enquête grand public sur la perception des risques.
4. Préparation d'un questionnaire sur la perception des risques destiné aux experts.
5. Impact de Tchernobyl dans l'opinion.

III. ETAT D'AVANCEMENT

1. Bibliographie

L'analyse bibliographique s'est poursuivie parallèlement aux travaux en cours. Elle concerne plus particulièrement le domaine de la communication.

2. La perception des situations à risque chez les travailleurs

Analysée une première fois en Novembre 1984 [1,2,3] chez les travailleurs de Saclay, elle l'a été une seconde fois en Mars 1987 [4]. Cette seconde enquête avait pour objectifs de contrôler les résultats obtenus antérieurement et de voir quelles pouvaient être les réactions d'un public "averti" face à des accidents comme celui de Tchernobyl.

S'agissant de la perception des situations à risque, le tableau suivant permet de comparer les résultats des deux enquêtes :

	SAC 1987		SAC 1984	
Libellé	Dang.	Non dang.	Dang.	Non dang.
Brancher un app. électrique près d'un point d'eau	74 %	6 %	72 %	7 %
Fumer	62 %	7 %	57 %	11 %
Vivre près d'un centre de stockage de déchets chimiques	58 %	10 %	56 %	11 %
Travailler dans une mine de charbon	55 %	9 %	54 %	8 %
Boire de l'alcool pendant la journée	54 %	12 %	54 %	14 %
Vivre à proximité d'une usine chimique	51 %	11 %	39 %	18 %
Passer à la radiographie souvent	49 %	13 %	52 %	11 %
Travailler en hauteur	44 %	9 %	51 %	9 %
Utiliser des produits chimiques	44 %	11 %	43 %	11 %
Utiliser des produits radioactifs	42 %	16 %	44 %	14 %
Travailler dans une usine de retraitement	39 %	17 %	39 %	18 %
Circuler en voiture	35 %	16 %	26 %	25 %
Preparer-conditionner des sources de radioactivité	25 %	24 %	41 %	20 %
Travailler dans une mine d'uranium	34 %	25 %	36 %	24 %
Vivre près d'un stockage de déchets radioactifs	29 %	40 %	37 %	31 %
Travailler quand il y a du bruit	23 %	28 %	25 %	26 %
Participer à l'expédition et au transport de produits radioactifs	21 %	38 %	23 %	38 %
Prendre des medicaments	17 %	37 %	17 %	34 %
Travailler en boite à gants	12 %	52 %	13 %	51 %
Travailler en zone contrôlée	11 %	50 %	13 %	50 %
Vivre à proximité d'une centrale nucléaire	11 %	64 %	8 %	72 %
Circuler en voiture sur le Centre	10 %	65 %		
Travailler dans une centrale thermique	8 %	61 %	8 %	69 %
Travailler dans une centrale nucléaire	7 %	68 %	6 %	70 %
Bricoler chez soi	4 %	67 %	5 %	69 %
Travailler devant un écran (ordinateur....)	4 %	77 %	5 %	70 %
Travailler avec des télémanipulateurs	4 %	78 %	5 %	75 %
Prendre l'avion	4 %	82 %	4 %	81 %

Il n'y a pas de changements importants dans la perception des situations à risque : "Brancher un appareil électrique près d'un point d'eau" et "Fumer" sont toujours les plus grands des risques. "Vivre à coté d'une centrale nucléaire" et "Travailler dans une centrale nucléaire" restent deux situations considérées comme peu dangereuses : les individus ne sont que 11 et 7 % respectivement pour les juger dangereuses. Cette appréciation basse du risque nucléaire par rapport aux autres apparait aussi dans le fait que "Vivre près d'un centre de stockage" est jugé dangereux par 58 % des agents s'il s'agit de déchets chimiques et par 29 % des agents s'il s'agit de déchets radioactifs. De même, travailler dans une mine de charbon est jugé comme plus dangereux que travailler dans une mine d'uranium (55 % et 35 % respectivement). Parmi les activités nucléaires, l'utilisation et le retraitement de produits radioactifs apparaissent comme les plus dangereux alors que le transport de produits radioactifs et la centrale nucléaire sont considérés comme les moins dangereux.

Globalement deux facteurs interviennent : l'un correspond à une inquiétude générale qui se manifeste face à tout risque, ou bien peut-être et plus simplement l'aversion pour le risque ; l'autre conduit les agents à singulariser les situations où le risque radioactif est présent. Les agents travaillant en robotique, biologie, informatique ou effectuant des tâches administratives, et qui ne sont donc pas exposés aux risques chimiques ou nucléaires, considèrent les activités à caractère chimique ou nucléaire comme plus dangereuses que ne le font ceux qui sont directement concernés : techniciens et ingénieurs en génie nucléaire, personnel oeuvrant dans les domaines de la santé, de la sécurité et de la protection. Une connaissance minimale des risques par la majorité des agents et la pratique journalière des risques pour un certain nombre d'entre eux, expliquent les résultats obtenus et notamment la relativisation du risque nucléaire par rapport aux autres risques.

Signalons pour finir deux faits majeurs : le sexe et le niveau d'étude interviennent dans la perception du danger : face aux risques, les femmes expriment toujours plus d'inquiétude que les hommes et il en est de même pour les non-cadres comparativement aux cadres.

Dans leur perception des accidents et de celui de Tchernobyl tout particulièrement, les travailleurs de Saclay ont généralement des avis plus modérés que les individus du public : ils croient moins à l'éventualité d'un tel accident en France (32 % au lieu de 54 % environ dans la population) ; ils sont plus nombreux à estimer que les conséquences en France de l'accident ont fait l'objet d'une dramatisation excessive (39 % alors qu'ils ne sont que 25 % à le penser dans le public). Les explications des experts leur paraissent un peu plus compréhensibles : cela est vrai pour un agent sur trois à Saclay mais seulement pour un individu sur cinq dans le public. La connaissance, ou pour le moins l'expérience du travail dans un centre d'études nucléaires, semble pouvoir expliquer de tels avis. Le personnel ne mésestime cependant pas l'importance de l'accident de Tchernobyl qui reste bien pour lui la première des catastrophes devant Bhopal et le SIDA.

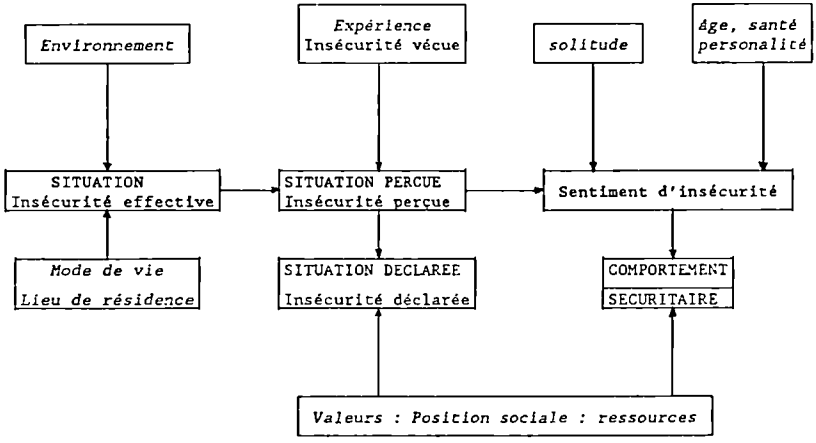
En tant qu'agent du CEA, ils ont, pour un sur deux environ, été interrogés sur Tchernobyl par leurs proches et font globalement confiance à l'institution pour connaître la vérité, ce qui concorde bien avec une logique de représentation.

3. La perception des situations à risque dans le grand public

Le rapport final de l'enquête nationale réalisée par l'IFOP en Février 1986 auprès de 1000 individus recueillis par quotas dans la population française est disponible [5]. Comme chez les travailleurs, l'aversion pour le risque constitue une dimension essentielle où interviennent plus ou moins les facteurs âge, sexe, diplôme, revenu et habitat. Les facteurs idéologiques et politiques ne sont pas absents et concernent plus particulièrement les risques industriels. Le champ des activités à risque se structure en sous-champs : l'industrie, le transport, le médical, le domestique, les loisirs,..... pour lesquels les critères d'appréciation du risque manifestement diffèrent.

De leur côté, les pratiquants d'une activité à risque la perçoivent toujours comme moins dangereuse que les individus extérieurs. De plus, percevoir un domaine d'activité comme dangereux va de pair avec le fait de douter de l'organisation de la sécurité dans le domaine. Enfin, tout accident technologique majeur a un impact négatif immédiat non seulement sur l'image du domaine qui le concerne mais aussi sur celles des domaines où de tels accidents sont susceptibles de se produire ; à ce titre, l'impact de Tchernobyl dans l'opinion est resté fort en 1987.

Le graphe d'influence suivant décrit la relation telle qu'elle semble s'effectuer entre insécurité effective et comportement sécuritaire.



Ces travaux ont été publiés dans [6] et exposés au récent colloque ACADI sur "La Maîtrise des risques technologiques" [7].

4. Questionnaire pour l'évaluation des risques par les experts

Ce questionnaire à remplir soi-même sera adressé à des experts de la gestion des risques qui oeuvrent dans les domaines nucléaire, chimique, environnemental, agronomique et médical. Il est prévu de contacter au moins une centaine d'experts. Le questionnaire, qui n'est pas encore finalisé, aborde pour un ensemble d'activités à risques les points suivants : nature de la situation ou de l'évènement qui génère le risque, nature des conséquences, attitudes de l'individu face au risque, organisation de la sécurité ; il s'agit de cerner finalement la notion d'acceptabilité.

5. Impacts socio-économiques de Tchernobyl :

L'impact sur l'opinion française de l'accident de Tchernobyl et des débats qui l'ont suivi, a été mesuré grâce aux enquêtes réalisées périodiquement dans le grand public. Un rapport [8] détaille les opinions sur le nucléaire et leurs évolutions de Mars 1977 à Juin 1987. On peut considérer qu'il n'y a plus en France une majorité pour approuver l'expansion du programme nucléaire civil. Par contre le soutien aux réalisations existantes se maintient et il n'y a pas de remise en cause des actions engagées qui seront terminées dans les années qui viennent.

ENQUETES	
J85	Juin 1985
CEA F86	Janvier-Février 1986
J86	Juin-Juillet 1986
F87	Janvier-Février 1987
J87	Juin 1987

LIBELLES	% CONTRE				
	J 85	CEA F 86	J 86	F 87	J 87
Après Tchernobyl, il faut fermer les centrales nucléaires françaises.			60	72	61
Il faut continuer à construire des centrales nucléaires	45	52	59	56	61
La construction des centrales nucléaires a été une bonne chose	31	25	41	32	38
La force de frappe est indispensable	32	28	34	31	29
Les spécialistes du nucléaire sont des gens très sérieux	13		13	9	10

Les dimensions conflictuelles du nucléaire ont été présentées lors d'un colloque qui s'est tenu à Paris en Juin 1987 consacré au nucléaire et aux problèmes de communication [9].

L'analyse de la presse traitant de l'accident de Tchernobyl a été entreprise. Un rapport [10] regroupe trois synthèses portant sur les conséquences socio-politiques de l'accident en RFA et en Grande Bretagne ainsi que sur le montant des indemnisations connues accordées au secteur agricole en Europe de l'Ouest. Ce document est plus orienté sur le politique et ne traite que de deux pays alors que H. OTWAY à ISPRA dans un travail analogue basé sur la presse a abordé les diverses conséquences qui ont été observées dans sept pays d'Europe [11].

6. Réunions

. ISPRA Septembre 1987 avec OTWAY H. (CCR)
. ROME Octobre 1987 avec CONVERSANO R. (ENEA)

IV. OBJECTIFS POUR L'ANNEE 1988

1. Poursuite des travaux bibliographiques

2. Colloque Nice 1988. Communications sur :a) les différents modèles de perception des risques ;b) le risque nucléaire parmi les risques industriels : quelles perceptions ?

3. Perception des risques par les experts : passation et traitement de l'enquête.

4. Suivi de l'impact de Tchernobyl dans l'opinion et conséquences socio- politiques de l'accident : poursuite des études de cas (France et Italie).

V. AUTRE GROUPE DE RECHERCHE COLLABORANT AU PROJET

Groupe de Recherche Energie, Technologie et Société (GRETS)
Direction des Etudes et Recherches Electricité de France
30, rue de Condé 75006 PARIS

VI. Références

[1] BASTIDE S., MOREAU A.
Enquête sur la perception de la sécurité et ses intervenants par le personnel du Centre d'Etudes Nucléaires de Saclay.
Rapport de synthèse + 4 Annexes, Rapport DPS 86/08, Octobre 1986.

[2] BASTIDE S., UZZAN K., MOREAU A.
Enquête sur la perception de l'accident de Tchernobyl par le personnel du Centre d'Etudes Nucléaires de Saclay, Rapport principal + annexe, Note LSEES 87/26, Octobre 1987.

[3] BASTIDE S., MOREAU A.
Enquête sur la perception de la sécurité par le personnel du Centre d'Etudes de Saclay - RGN 1986, n° 6, p.536-542.

[4] BASTIDE S., MOREAU A.
La sécurité, comment l'entendez-vous ? Les Echos du Groupe CEA, 1987, n°6, p.9-12.

[5] BASTIDE S., CARDE C., PAGES J.P.
Perception des risques. Enquête de Février 1986 - Rapport Final. Note LSEES 87/01.

[6] BASTIDE S., PAGES J.P.
Perception des risques et communication. Approche et premiers résultats in : La société vulnérable. Evaluer et maitriser les risques. Presses de l'Ecole Normale Supérieure, 1987, p.93-110.

[7] BASTIDE S., PAGES J.P.
De la gestion des risques au risque perçu - Colloque international "La Maitrise des risques technologiques", UNESCO-PARIS, Décembre 1987.

[8] BARNY M.H., BONNEFOUS S., PAGES J.P.
Le nucléaire et l'opinion publique quatorze mois après Tchernobyl - Note LSEES 87/16, Août 1987.

[9] ANSEL Ph., BARNY M.H., PAGES J.P.
Débat nucléaire et théorie de l'opinion. L'approche de l'opinion publique en France - Actes du Colloque SFEN "Energie nucléaire, communication et opinion publique", Paris, Juin 1987, RGN 1987, n°5, p.451-459.

[10] CHAUSSE J.
L'accident de Tchernobyl : quelques conséquences économiques et sociales - Note LSEES 87/17, Septembre 1987.

[11] OTWAY H., et al.
An Analysis of the print media in Europe following the Chernobyl accident - CEC Ispra, EUR 11043 EN,1987.

RADIATION PROTECTION PROGRAMME
Final Report

Contractor:

Contract no.: BI6-F-122-F
Sub Contract SC-001-F

Université Paul Sabatier
Division des Affaires Scientifiques
118, Route de Narbonne
F-31062 Toulouse Cedex

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

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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 DETRIMENT OBJECTIF GLOBAL ET DE SON COUT EN RELATION AVEC LES CONSIDERATIONS ECONOMIQUES - RECHERCHES SUR LE VEGETAL

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:

- Réglage du dispositif construit pour évaluer la mutagénicité globale de configurations variées d'environnement. Premières expériences in situ.

- Evaluation de la mutagénicité d'atmosphères faiblement enrichies en gaz polluants :

  - analyse et exploitation des données obtenues en laboratoire pour évaluer la mutagénicité du SO_2 ;
  - premiers développements techniques pour évaluer la mutagénicité des NO_x.

- Evaluation de la mutagénicité du Radon : mise au point d'un dispositif expérimental et première expérience.

- Evaluation de la mutagénicité de faibles irradiations internes.

- Caractérisation de la relation dose-effet pour de faibles doses d'irradiations réalisées au moyen d'une source de Co 60.

III. Progress achieved:

Remarques préliminaires :

- Le contrat de recherche, en principe terminé au 01-07-1987, a été prolongé pour une période de deux ans. Le travail effectué au cours de la période 1985-1987 a fait l'objet d'un Rapport Final 1986-1987, lequel est cité au point VI. (Publications). En conséquence, le présent rapport est traité comme un Progress Report annuel.

- Toutes les expériences ont été réalisées à l'aide du marqueur génétique a_1-a_2 porté par la variété xanthi du Tabac (Nicotiana tabacum L.). Tous les détails concernant ce système et son utilisation ont été fournis dans des Rapports précédents.

-Evaluation de la mutagénicité globale de configurations variées d'environnement

La construction du dispositif a été terminée au début de 1987 (cf. Rapport Final 86-87 pour détails). Des essais de fonctionnement ont été effectués au laboratoire et une première expérience a été conduite en 1987 sur un site pollué de l'agglomération Toulousaine (France) ; il s'agit de la propriété DANIS située au voisinage de l'usine d'incinération des ordures ménagères du Mirail, dans le Sud-Ouest de l'agglomération. Des mesures physico-chimiques ont démontré que les polluants émis par l'usine ont bien atteint le dispositif expérimental. Il apparaît que la valeur moyenne du taux de réversion obtenue sur les plants soumis à l'atmosphère polluée du site est presque six fois plus grande que celle observée sur les plants témoins soumis à de l'air filtré. Il semble évident que cet effet spectaculaire est dû, d'une part, à l'action des polluants émis par l'usine et, d'autre part, à la pollution urbaine liée à la proximité de la Ville de Toulouse.

-Evaluation de la mutagénicité d'atmosphères faiblement enrichies en gaz polluants

- Expérimentation sur le SO_2

Quatre expériences ont été réalisées en cultivant des plants dans deux enceintes ayant les mêmes caractéristiques, à l'exception de l'air qui les traversait : l'une des enceintes était alimentée en air filtré, l'autre l'était par le même air filtré enrichi par des quantités connues de SO_2. Les résultats de l'expérience 1, réalisée pendant la mise au point de l'appareillage, n'ont pas été exploités car les teneurs en SO_2 ont trop varié (50 à 200ppb). Les expériences 2 et 3 ont été faites avec des concentrations de SO_2 fixées respectivement à 51 et 110 ppb. Dans l'expérience 4, réalisée avec 950 ppb, tous les plants traités ont succombé. On a donc procédé à l'analyse statistique de trente valeurs du taux de réversion du système marqueur : 18 en atmosphère filtrée, c'est-à-dire six dans chacune des expériences 1, 2 et 3, et six dans chacune des deux atmosphères polluées en SO_2 aux concentrations de 51 et 110 ppb. Ces données

préliminaires permettent de décrire, dans une plage de teneurs en SO_2 non létales, une relation dose-effet linéaire :

$$y = 0.231 + 0.014\ x \quad ; \quad 0 \leqslant x \leqslant 110 \text{ ppb},$$

où y est le taux de réversion, p x 10^5, du système marqueur.

-Evaluation de la mutagénicité du Radon

Deux lots de cent plantules chacun ont été transportés, au stade 1-2 feuilles, dans la chambre d'inhalation de Radon de la COGEMA à Razès (Haute-Vienne , France). Un lot a été soumis à une concentration de Radon égale à 1000 CMA, pendant que l'autre était protégé du gaz radioactif à l'aide d'un boîtier en plastique. De cette expérience préliminaire, on peut conclure que (i) la présence de Radon à de très fortes concentrations (jusqu'à 1000 CMA) ne contrarie pas le développement des plantules porteuses du système marqueur, (ii) le Radon a un effet mutagène évident puisque le taux de reversion observé sur les plants exposés est plus de 30 fois celui des témoins, et (iii) bien entendu, des investigations complémentaires avec de plus faibles concentrations sont nécessaires.

-Evaluation de la mutagénicité de faibles irradiations internes

Cinq lots de trente plants chacun ont été cultivés sur cinq substrats caractérisés par des teneurs variées en radionuclides naturels (uranium et ses descendants) : un substrat témoin (bruit de fond naturel) et quatre substrats caractérisés, à volume égal, par des radioactivités globales respectives de 2.4, 1.8, 1.2 et 0.6 x 10^{-3} cGy/h, toutes inférieures à la valeur seuil au-dessous de laquelle, en irradiation externe, aucune modification du système marqueur n'a été observée sur des plants cultivés au-dessus d'affleurements uranifères. Les taux de réversion obtenus sur les plants cultivés dans les substrats anormalement radioactifs sont significativement plus élevés que celui observé sur les plants témoins ; ils ne sont toutefois pas significativement différents entre eux. Sur la base de ces résultats, il semble raisonnable d'affirmer que les irradiations internes liées à l'absorption de radionuclides soient plus efficaces sur le système marqueur que les irradiations externes.

-Caractérisation de la relation dose-effet pour de faibles doses d'irradiations réalisées au moyen d'une source de Co 60

Cinq lots de plants ont été soumis à des doses de 0 (bruit de fond naturel), 0.134, 0.245, 0.369 et 0.494 cGy/h. Ce gradient a été obtenu en plaçant les boîtiers de culture à des distances variées de la source de Co 60 située dans la chambre d'irradiation de la Section de Toxicologie et Cancérologie Expérimentale de l'IPSN, DPS-SPE du CEA à Bruyères-le-Châtel, Essonne (France). L'analyse statistique montre que la réponse (taux de réversion x 105) est linéaire dans la plage de débits de dose expérimentés :

$$y = 3.42 + 16.58\ x \quad ; \quad 0 \leqslant x \leqslant 0.494 \text{ cGy/h}.$$

Toutefois, une analyse plus détaillée montre qu'il serait pratiquement

aussi raisonnable d'admettre une réponse de type cubique, en l'occurrence,

$$y = 3.09 + 20.36\ x - 16.00\ x^3.$$

Graphiquement, ces deux réponses sont très proches l'une de l'autre. Toutefois, la seconde, dont le point d'inflexion a pour coordonnées (0, 3.09), a une concavité tournée vers le bas dans la plage des débits de dose expérimentés. Ceci pourrait signifier que, si un seuil devait exister aux débits de dose bas, celui-ci serait de type ⌐‾ plutôt que de type —/. C'est ce qu'il va falloir rechercher.

IV. OBJECTIVES FOR THE NEXT 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 urbain, industriel et professionnel.

- 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 les NO_x.

- Evaluation de la mutagénicité du Radon : expérimentation avec des concentrations inférieures à 1000 CM.

- Caractérisation de la relation dose-effet pour de faibles doses d'irradiations réalisées au moyen d'une source de Co 60 : modification du dispositif d'irradiation et étude d'un gradient plus proche du point zéro.

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

- Association EURATOM-CEA, C.E.N. de Fontenay-aux-Roses, DPS-REI, BP N° 6, F-92265 Fontenay-aux-Roses Cedex

- 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, C.E.A., Bruyères-le-Châtel, Essonne, France (Dr H. METIVIER)

- Laboratoire de Chimie-Energie-Environnement de l'Ecole Nationale Supérieure de Chimie de Toulouse, France (Pr. L.TORRES)

- Laboratoire de Mutagenèse de la Station d'Amélioration des Plantes de l'INRA, F-21034 Dijon Cedex (Dr H. DULIEU)

- Département Environnement et Service Communal d'Hygiène et de Santé de la Ville de Toulouse, France (M.J.P. PONTHUS)

- Laboratoire d'étude de la pollution atmosphérique de Pau-Montardon, France (Dr J. BONTE)

- Société Technofroi, Avignon, France

VI. Publications :

DELPOUX M., M.A. DALEBROUX, H. DULIEU, A. LEONARD - L'irradiation naturelle tellurique et les êtres vivants - Sous presse in : *Bull. de la Soc. d'Ecophysiologie, 27 pp dactylographiées*

MURATET S., O. VERNET (en préparation) - Etude et comparaison des effets génétiques et biologiques de la pollution atmosphérique et des très faibles doses de radioactivité naturelle *Thèse d'Université, Université Paul Sabatier, Toulouse (France)*

MURATET S., O. VERNET, M. DELPOUX, M.A. DALEBROUX (en préparation) - Evaluation en laboratoire des effets génétiques du SO_2 sur le système $a_1^+/a_-\ a_2^+/a_-$ de *Nicotiana tabacum L.* var. *xanthi*.

TORRES L., M. HAZIZA, S. MURATET, O. VERNET, M. DELPOUX (en préparation) - Générateur d'atmosphères étalons pour l'étude de l'action de traces de polluants sur les végétaux

Contrat N° B16-0122-F(D), sous-contrat N° SC-001-F - Génotoxicité comparée des principaux facteurs de l'environnement - RAPPORT FINAL 86-87.

RADIATION PROTECTION PROGRAMME
Final Report

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-122-F
Sub Contract SC-002-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:

Analysis of adopted countermeasures in the different countries of the EEC following the Chernobyl accident.

List of projects:

1. Analysis of the adopted countermeasures in the different countries of the EEC following the Chernobyl accident.

Title of the project no.: 2

EVALUATION DU DETRIMENT OBJECTIF GLOBAL ET DE SON COUT EN RELATION AVEC LES CONSIDERATIONS ECONOMIQUES -
Analyse des contremesures adoptées dans les différents pays de la Communauté Européenne après l'accident de Tchernobyl.

Head(s) of project: F. FAGNANI

Scientific staff: F. FAGNANI
J. LOMBARD

I. Objectives of the project:

II. Objectives for the reporting period:

L'analyse des contremesures adoptées, suite à Tchernobyl, dans différents pays de la CEE afin de déterminer quels sont les principaux critères de choix qui sont intervenus dans chacun de ces pays.

III. Progress achieved:

1. Le rapport se fonde sur la méthode "PREF CALC" (PREFerences CALCulées) qui permet, à partir du classement d'un ensemble d'options alternatives définies selon plusieurs critères, de déterminer le poids respectif de chacun de ces critères compatible avec le classement proposé.

2. L'analyse a été faite pour cinq pays représentatifs des choix faits suite à Tchernobyl dans la CEE : l'Espagne, la France, le Royaume-Uni, l'Italie et un pays imaginaire qui aurait eu recours à la distribution de plaquettes d'iode.

Il ressort principalement que si l'on excepte le cas hypothétique de ce cinquième pays, le critère de coût, qui limite l'adoption de contre-mesures trop onéreuses, est le plus explicatif des choix réalisés par ces pays. Si l'on excepte l'Espagne, qui n'a pas adopté de contremesure particulière, il apparaît que le choix se justifie également soit par un souci de limiter le risque individuel maximal, soit au contraire, par d'autres considérations d'ordre psychologique liées à l'acceptabilité des contremesures et à leur capacité à répondre aux attentes du public. En revanche, le critère du risque collectif n'apparaît pas avoir joué un rôle significatif dans les décisions prises par ces pays de la CEE.

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

V. Publications:

J. LOMBARD - Analyse des contremesures adoptées, suite à Tchernobyl, dans différents pays de la CEE - Congrès Franco-Italien, AIRA/SFRP, Caltelgandolfo, Rome, 12-13 Octobre 1987

J. LOMBARD, F. FAGNANI - L'analyse des contremesures adoptées, suite à Tchernobyl, dans différents pays de la CEE : Aspects méthodologiques, Rapport CEPN, N° 125, Décembre 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor

Contract no BI6-F-123-D

Deutsches Krebsforschungszentrum
Heidelberg
Institut für Nuklearmedizin
Im Neuenheimer Feld 280
D-6900 Heidelberg 1

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

Prof. Dr. G. van Kaick
Institut für Nuklearmedizin
Deutsch.Krebsforsch.zentr.Heidelberg
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.

Titel of the project no: BIO-F-369-81-D

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. 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. Luhrs; Dr. I. Zuna
Statistical evaluation: Dr. H. Wesch

I. Objectives of the project:

The aim of the German Thorotrast Study is: to study the late effects of incorporated colloidal Thoriumdioxide by epidemiological observation and clinical and biophysical examination of the patients; to compare the results with 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 of members of the control group
- Statistical evaluation of the epidemiological, clinical and biophysical data

III. Progress achieved:

Clinical results:
During the last year we performed 142 outpatient examinations, of which 77 were on Thorotrast patients and 65 on control patients. Eight patients were examined two times during the year because of the suspicion of a developing liver cancer. Table 1 summarizes selected diagnostic results of the two groups. Note the similar distribution with regard to diseases not associated with Thorotrast. This supports the validity for making statistical comparisons between the Thorotrast patients and the patients making up the control arm in our study.

Table 1: Selected diagnostic findings of the out-patient examination in 1987

Findings	Thorotrast (n=69 patients)	Control (n=65 patients)
Solid liver tumor	5	0
Cystic lesions in the liver	6	5
Diffuse parenchymal liver disease	47	29
Tumor of the spleen	1	0
Alterations of the peripheral blood picture	15	7
Solid renal tumor	0	1
Renal cysts	9	10
Adenoma of the prostate	10	12
Thyroid goitre	17	13
Gall stones	10	9
Diabetes mellitus	7	18
Hypertension	15	29
Paravascular ThO_2-deposits	21	0

Epidemiological results:
Causes of death of 45 patients who have died during 1987 could be clarified. The proportion of deaths attributed to liver cancer (n=6) compared to deaths from other diseases decreased in the Thorotrast group compared to prior years. In addition, there were three more cases of external bile duct carcinomas and two more cases of plasmocytomas which appeared only in the Thorotrast group. One of the Thorotrast patients died about 8 years after resection of a primary liver cancer by neoplastic liver disease.
During the last year we reevaluated the patient data sheets to uncover possible errors in patient selection and classification. Thus 50 patients of the non examined Thorotrast group were excluded from the study as the administration of Thorotrast was very questionable. This as well as other minor changes were made which may alter the sum of the former numerical values but which in no way changes the reported trends and findings.

Table 2: Final fate of the patients

Selected diseases	Thorotrast (deceased 2103 of 2327)	Control (deceased 1458 of 1890)
Liver cancer	393 (16.9)	2 (0.11)
Ca. of extrahep. bile ducts	23 (0.94)	6 (0.32)
Liver cirrhosis	299 (12.8)	42 (2.22)
Myeloproliferative disease	35 (1.50)	3 (0.16)
Chron. lymph. leukaemia	3 (0.13)	3 (0.16)
Bone marrow failure	25 (1.07)	1 (0.05)
Hodgkin's lymphoma	3 (0.13)	2 (0.11)
Non-Hodgkin's lymphoma	16 (0.68)	7 (0.37)
Plasmocytoma	7 (0.30)	1 (0.05)
Bone sarcoma	4 (0.17)	1 (0.05)
Ca. of the larynx	5 (0.21)	1 (0.05)
Lung cancer	47 (2.02)	43 (2.28)
Pleural mesothelioma	3 (0.13)	0 (-)
Pancreatic carcinoma	18 (0.77)	5 (0.26)
Ca. of the G.I. tract	58 (2.49)	74 (3.92)
Kidney cancer	7 (0.30)	6 (0.32)
Prostatic cancer	16 (0.68)	11 (0.58)

( )= % related to the total number of patients

Though the total number of lung cancers is not different between the Thorotrast and control group the curves of the cumulative death rate cross after about the 30th year of exposure (Figure 1).

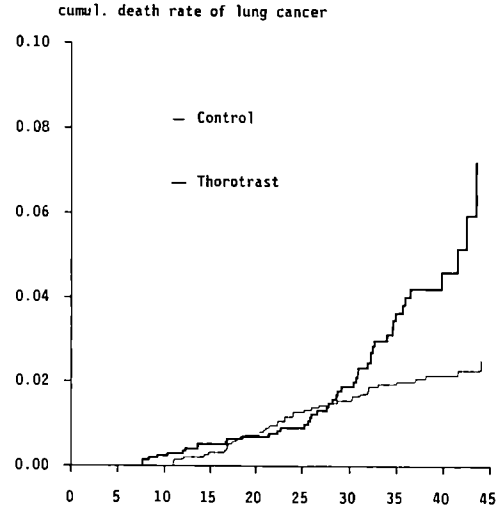


Figure 1

The cumulative death rate of lung cancer is calculated by putting each case of lung cancer in relation to the number of individuals still at risk. This fraction is summed up over all the events of lung cancer. That means we will have to observe the final fate of all the patients to determine if there is a higher incidence of lung cancer in Thorotrast patients after more than three decades of exposure to the exhaled Thoron.

IV. Objectives for the next reporting period:

The working program will be continued according to the recommendations of the coordinating committee

- regular correspondance with about 800 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 belonging 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 examination 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, School of Medicine, Division of Radiobiology, Univ. of Utah, Salt Lake City, U.S.A. (member of the coordination committee of the German Thorotrast study)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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

Development of fundamental data for radiation protection

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:

The Commission met at Washington DC in March; and at Como, Italy in September together with its four expert committees and observers from international organisations with interests in radiation-induced health effects and in the implementation of the ICRP's recommendations.

Three reports from committees were adopted and are being published in the Annals of the ICRP, together with reports adopted at a previous meeting.

In addition, an addendum (part 4) to ICRP Publication 30 (Limits for intakes of radionuclides by workers) was approved, in which revised ALI's and DAC's are given for actinides. This revision is necessary because of a recommendation to use new metabolic parameters for the actinides as proposed in ICRP Publication 48 (The metabolism of plutonuim and related elements). The addendum will also give ALI's and DAC's for ^{82}Sr, ^{95m}Tc, ^{95}Tc ^{116}Sb and ^{134}La for which transformation data were given in ICRP Publication 38 (Radionuclide transformation; energy and intensity emissions).

Four Task Groups were formed in 1987 whose objectives were to:-

- revise the basic recommendatons

- provide an update on risk estimates for cancer and genetic effects

- revise ICRP Publication 40 (Protection of the public in the event of major radiation accidents, principles for planning)

- advise on the biological basis for dose limitation in the skin.

These Task Groups are expected to provide data for use in the revision of the basic recommendations.

In addition, a Working Group was formed to revise the definitions of concepts and quantities as an input into the revision of the basic recommendations; and a Working Party was formed to compare organ masses using anthropomorphic phantoms as an input into the Task Group revising ICRP Publication 23 (Reference Man).

Actions resulting from committee reports were:

- a statement on proposed changes in f_1 values and retention half-lives in liver and bone on ALI's of some actinides

- a statement on radon relating to exposure of patients, workers and the recreational use of spas

- a statement on reference terms for estimates of radiation dose for X-ray mammography.

Of particular interest at the Como meeting were recent publications from the Radiation Effects Research Foundation on the Japanese Life Span Study. Committee 1 evaluated a paper by Preston and Pierce (The effect of changes in dosimetry on cancer mortality risk estimates in the atomic bomb survivors RERF TR-9-87) and reports from committee members on age-related cancer risks for particular types of cancer. Young people (<10 years of age) are more at risk than older persons; but the Committee concluded that the risk data are as yet far from conclusive. The Commission therefore decided to await the result of the comprehensive evaluation of its sources of epidemiological information (eg UNSCEAR and BEIR) and the views of its own Task Group on these data before judging the consequences for the revision of its system of dose limitation.

In the meantime, it will be prudent to follow the Commission's present recommendations on dose limitation as they were intended to be interpreted. When this is done the value of the dose limits, in most cases, will not be the controlling factor in the restriction of dose. Thus the Commission considers that the final judgement on the choice of dose limits can await full scientific review without any serious consequences.

Committee 1 were also informed of the possibility that a threshold may exist for the occurrence of serious mental retardation in children exposed while in utero, particularly in the 8-15 week post-conception period. Confirmation of the presence or absence of a threshold is potentially important for radiation protection purposes, the present position being that no threshold is assumed.

Task Groups reported satisfactory progress on several topics with expected dates for completion:

- RBE values for non-stochastic effects(1988/89)
- respiratory tract models (1988)
- revision of ICRP Publication 40 (1988/89)
- revision of ICRP Publication 23 (1989/90)
- age-dependent dosimetry and dose per unit intake for members of the public (1989)
- protection of medical workers (1988)
- the use of techniques other than cost benefit analysis for optimisation (1989)
- revision of the basic recommendations (1990/91).

The Commission will meet in October 1988 and its Committees and Task Groups will meet independently as appropriate.

IV. Objectives for the next reporting period:

To continue the work in progress as defined in Section III. It is anticipated that the revised basic recommendations will be published at the end of this quinquennium.

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

Nil

VI. Publications:

ICRP Publication 50. Lung cancer risk from indoor exposure to radon daughters. Ann. ICRP 17, No. 1, 1987.

ICRP Publication 51. Data for use in protection against external radiation. Ann. ICRP 17, Nos. 2-3, 1987.

ICRP Publication 52. Protection of the patient in nuclear medicine. Ann. ICRP 17, No. 4, 1987.

ICRP Publication 53. Radiation dose to patients from radiopharmaceuticals. Ann. ICRP 18, Nos. 1-4, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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, A.C. Engels 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:

The absorbed dose distributions obtained with polymethylmethacrylate (pmma) phantoms of various thicknesses are compared to the dose distributions in BR-12 (breast simulating material) A-150 plastic and fat phantoms. Information is obtained on the distribution of the thickness of compressed breasts and on focal charge per mammogram in a screening program. Combined with the focal charge and dose measurements for pmma phantoms of various thicknesses this results in absorbed dose estimates for the female breast. A protocol is formulated for quality assurance in mammography and is being applied and tested at the Comprehensive Cancer Centre Rotterdam.

III. Progress achieved:

Absorbed dose measurements have been made with a BF2571 ionization chamber in phantoms of various materials with a surface area of about 100 mm x 100 mm and a thickness of about 5 cm employing a displacement correction factor slightly varying with depth and phantom material. The phantom materials employed were polymethylmethacrylate (pmma), BR12 (a special material simulating the average breast, White and Constantinou, p. 132. In: Progress in Medical Radiation Physics. Vol. 1, Plenum Press, 1982), A-150 plastic (a material simulating muscle tissue) and fat (simulating the fatty tissue in the breast). The depth dose distributions at the central beam axis measured in the phantoms placed at a source-to-surface distance (SSD) of 526 mm for irradiation with 31 kV X rays (first half value layer (HVL) : 0.33 mm Al; homogeneity factor (H): 0.80) are given in the figure.

There is a strong dependence of the steepness of the depth-dose curve on the phantom material. The steepest depth dose distribution is found for A-150 plastic (which might represent glandular tissue). The shallowest depth-dose curve is found for fat, whereas the results for pmma and BR12 are intermediate and only marginally different.

The use of pmma as phantom material seems reasonable since the results are similar to those for BR12 and intermediate with respect to the materials composing the breast (glandular and fatty tissue). Therefore, the dose measurements made for pmma phantoms of various thicknesses (previous progress report) can be used for comparison with results obtained with real mammograms.

At the mammography unit of the Comprehensive Cancer Centre Rotterdam (IKR) a mAs meter has been installed which allows determinaton of the focal charge per radiograph. From the depth-dose and focal charge measurements with pmma phantoms a relation between entrance dose and mAs value could be derived as a function of SSD and tube voltage. This allows also to derive the dose distribution and average absorbed dose as a function of SSD, phantom thicknes and tube voltage from the focal charge per radiograph.

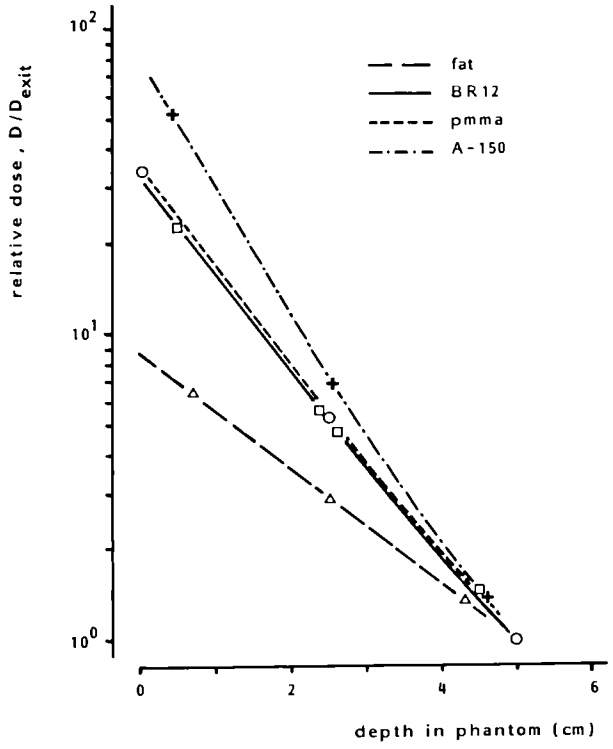
By using the mAs meter it is also possible to determine the focal charge during real mammography. When pmma would be a suitable phantom material to simulate the compressed breast, the readings of the mAs meter for equal thicknesses of pmma phantoms and compressed breasts should be similar.

At the IKR, the compressed breast thicknesses (subdivided in classes with a range of 1 cm) and the focal charge values have been determined for 700 mammograms (see Table). The average compressed breast thickness and standard deviation is (50 ± 13) mm. Also given in the table are the ranges of the mAs-values for pmma phantoms for the various thickness classes. A comparison of the average mAs values for the compressed breasts with the ranges of mAs values for pmma phantoms in the various thickness classes indicates that for the approximately average breast thicknesses the values are similar. For thinner compressed breasts the mAs values are larger than those for pmma phantoms, whereas for thicker breasts the mAs values are smaller than those for pmma phantoms.

This indicates that with regard to dosimetry pmma is a reasonable phantom material for simulating the breast at the average compressed thickness, but not for thin and thick compressed breasts. This observation is most likely related to a variation in the tissue composition of the breasts as a function of their compressed thickness. For thicker and thin-

ner compressed breasts, the percentages of fatty tissue and glandular tissue might be different from those in the average compressed breast. Fat and muscle tissue show considerable differences in attenuation (see figure). A higher percentage of fatty tissue in thicker breasts and a higher percentage of glandular tissue in thinner breasts would result in respectively lower and higher doses compared to the average breast composition (pmma).

Also given in the table are the estimated average absorbed dose values for actual mammograms which are for thin breasts higher than, for average breasts similar to and for thick breasts lower than expected on the basis of pmma phantom measurements.



Figure

Central axis depth-dose curves in 50 mm thick phantoms of various materials irradiated with 31 kV X-rays (first HVL: 0.33 mmAl; H = 0.80) at an SSD of 526 mm.

TABLE

COMPARISON OF mAs VALUES FOR RADIOGRAPHS OF COMPRESSED BREASTS WITH THOSE FOR PMMA PHANTOMS AT VARIOUS THICKNESSES AND ESTIMATED ABSORBED DOSES FOR ACTUAL MAMMOGRAMS

Tube voltage (kV)	Thickness, d_m (mm) of compressed breast or pmma phantom	number* of actual mammograms per thickness class	mean focal charge for actual mammograms and standard deviation (mAs)	range of focal charge, E_p for radiographs of pmma phantoms (mAs)	estimated average absorbed dose per real mammogram (mGy)
28	$d_m < 20$	8	7.4 ± 1.9	$E_p < 5.3$	0.28
28	$20 < d_m < 30$	25	10.3 ± 3.0	$5.3 < E_p < 8.2$	0.33
28	$30 < d_m < 40$	130	13.0 ± 4.0	$8.2 < E_p < 13.9$	0.38
28	$40 < d_m < 50$	292	16.7 ± 5.3	$13.9 < E_p < 22.3$	0.43
31	$50 < d_m < 60$	167	12.0 ± 3.0	$14.0 < E_p < 20.1$	0.44
31	$60 < d_m < 70$	58	13.8 ± 3.5	$20.1 < E_p < 31.2$	0.48
31	$70 < d_m < 80$	27	15.0 ± 3.5	$31.2 < E_p < 45.1$	0.51
31	$80 < d_m < 90$	11	17.9 ± 4.2	$45.1 < E_p < 64.0$	0.59
31	$90 < d_m$	5	19.2 ± 7.2	$64.0 < E_p$	0.63

* not representative for distribution of compressed breast thicknesses

IV. Objectives for the next reporting period:

The deviation in compressed breast tissue composition from the average composition (simulated by pmma and BR12) will be investigated by estimations of percentages of glandular tissue and mAs measurements for mammograms of compressed breasts. The quality assurance protocol will be continued to be tested at the Comprehensive Cancer Centre Rotterdam. Investigations of film processing and on doses required for new types of film/screen combinations will be continued. An analysis of screen/film mammography in terms of information theory will be made.

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

Dr. 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.

VI. Publications:

J. Zoetelief and J.J. Broerse. Dosimetry and quality assurance of physical aspects in mammography. Proceedings of the 8th ICRR, Vol. 1. B31, Edinburgh, 1987.
J. Zoetelief, A.C. Engels, N.J.P. de Wit and J.J. Broerse. Fysische aspecten van film/scherm mammografie in grootschalige programma's voor vroegtijdige opsporing van borstkanker. Gamma, 10, 278-286, 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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, Associated Professor of Clinical Physiology, University of Pisa.

Scientific staff:

Distante A., M.D., Parodi O., M.D., Neglia D., M.D., Levorato D., M.D., Arlotta C., M.D., Marzullo P., M.D., Picano E., 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:

The patients who developed a deterioration of myocardial function detected by non invasive methods underwent cardiac catheterization. In this subgroup of patients angiographic data were intended to provide a standard for the assessment of sensitivity and specificity of the non invasive methods. The stable patients were followed by 2D-Echo and RNA serial studies as a basis to assess to predictory value of each method.

III. Progress achieved:

We continued the followup of the cardiomyopathy patients that were considered stable on the basis of Doppler echocardiogram. These patients undergo echocardiographic study (ECHO) every 6 months and radioisotopic angiography (RNA) with 99-Tecnetium yearly. Abnormalities recorded at ECHO determine the requirement of a complete hemodynamic study with cardiac catheterization and angiography (CCA). During the last year we performed such a CCA study in 26 of these patients, with endomyocardial biopsy in 19. Of these patients 23 were also studied with Radionuclide Angiography (RNA).

Results of cardiac catheterization

Cardiac output, pressures, systemic and pulmonary vascular resistances were within normal limits in all the patients studied. Coronary arteries were also normal in all cases, so that we could definitely exclude a coronary artery disease in these patients (previously evaluated with exercise-stress test and 24-hour Holter monitoring before entering the study).

The results of angiographic study (CCA) of volumes and regional dyssynergies were compared with the results of the non invasive assessment (ECHO and RNA). The results are presented in Table 1.

Table 1

COMPARISON OF CARDIAC CATHETERIZATION RESULTS VS 2D-ECHO AND RNA

	2d-ECHO 24 PATIENTS		RNA 23 PATIENTS	
	ACC	DISC	ACC	DISC
RV ENLARGEMENT	15 62%	9 38%	13 54%	10 46%
RV DYSSYNERGIES	16 67%	8 33%	12 52%	11 48%
LV ENLARGEMENT	21 88%	3 12%	20 87%	3 13%
LV DYSSYNERGIES	17 71%	7 29%	18 78%	5 22%

ACC = Accordance
DISC = Discordance

Comparing echocardiographic and radioisotopic results with ventricular contrastographic data, we found that the invasive method (CCA), initially assumed as the reference approach, was in fact less sensitive both of ECHO and RNA. Accordance was good as far as left volumes and dyssynergies are concerned (87% and 78% for enlargement and dyssynergies respectively at ECHO, vs. 87% and 78% at RNA) while it was less evident in the analysis of the right ventricle (62% and 67% for enlargement and dyssynergy with ECHO, vs. 54% and 52% with RNA).
The low sensitivity and specificity of the contrastographic analysis may be due to the use of standard and not "ad hoc" projections, to the injection of contrast medium in the right atrium and not in the ventricle, and to the difficulty of a complete analysis of the right ventricle because of its abnormal geometric shape.

Table 2

ENDOMYOCARDIAL BIOPSIES RESULTS

TOTAL	19
NORMAL	2
ABNORMAL	17

ABNORMAL	LV	RV
Nuclear hyperthrophy	3	1
Cellular hyperthrophy	14	5
Interstitial fibrosis	10	2
Lipomatosis	1	2
Infiltrates	9	1

Endomyocardial biopsy results are listed in Table 2: it showed aspecific alterations but, it was anyway a reliable index of disease (17 abnormal bioptic findings in 19 studied patients). In fact the same aspecific abnormalities were found in advanced dilated cardiomyopathies. It is thus necessary to increase the number of biopsies to obtain further informations. On the basis of the comparison with angiography, we can conclude that echocardiography and radioisotopic angiography seem to be the best method to diagnose and follow-up patients with initial myocardial damage.

IV. Objectives for the next reporting period:

In the next reporting period, a quantitative comparison between the results obtained with the three methods will be made, either for the volumes or the regional dissynergies, with statistical evaluation. New Patients will be recruited; of those already in the study population, the stable ones will be followed up, while those whose cardiac performance will worsen will be studied invasively.

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

C.N.R. - Institute Clinical Physiology

VI. Publications:

Le cardiomiopatie destre: caratterizzazione delle aritmie mediante elettrocardiografia dinamica.
M. Piacenti, D. Levorato, C. Arlotta, S. Berti, D. Neglia, M.G. Bongiorni, L. Paperini, C. Contini.
Atti del Congresso "Elettrocardiografia dinamica in Italia. 10 anni di esperienze", Pisa 8-10 giugno 1987.

Aritmie cardiache e cardiomiopatie. Esistono differenze fra forme iniziali e conclamate?
C. Arlotta, D. Levorato, M. Piacenti, M.G. Bongiorni, A. Pozzolini, M.T. Baratto, C. Contini.
Atti del Congresso "Elettrocardiografia dinamica in Italia. 10 anni di esperienze", Pisa 8-10 giugno 1987.

Ventricular arrhythmias in the healthy subjects. Are tney a spy of an early myocardial damage?
C. Contini, D. Levorato, M.G. Bongiorni, M.T. Baratto, C. Arlotta, M. Piacenti, A. Pozzolini, L. Paperini, G. Kraft.
The "new frontiers" of arrhythmias 1988, Marilleva, January 31 - February 6, 1988 (invited paper).

Title of the project no.: 2

Diagnostic efficacy of immunoscintigraphy with radioactive monoclonal anti- bodies (versus bidimensional echography).

Head(s) of project:

G. Mariani, Associated Professor of Medical Pathophysiology, University of Pisa

Scientific staff:

Bianchi R., M.D., Bellina C.R., M.D., Guzzardi R., D. Phys., Rosa C., M.D., Mazzuca N., M.D., Molea N., M.D.

I. Objectives of the project:

1) To evaluate the patients exposure to ionizing radiation involved by the use of a newly developed diagnostic procedure, that is, radioimmunoscintigraphy 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 assess the organ doses resulting from the use of labelled monoclonal antibodies for immunoscintigraphy.

To assess the comparison of these radiation exposure with the diagnostic efficacy of immunoscintigraphy, in comparison with other conventional non invasive techniques in patients with adenocarcinoma.

III. Progress achieved:

A recently completed multicenter clinical trial was aimed at evaluating the efficacy of monoclonal anti-CEA F023C5-F (ab') as an immunoscintigraphy (ISG) agent for the detection of tumor lesions.

The study (which was coordinated by the National Research Council of Italy) involved a total of 542 patients, and the monoclonal tracer was labelled with either Iodine131 (preferentially for tumors located in the abdominal area) or Indium-111 (preferentially for tumors located above the diaphragm). The main findings of the study were the high proportion of tumor lesions detected at ISG (over 80% of the localizations in CEA-seropositive patients) including a high number of unexpected lesions a relevant fraction of which occurred in patients previously classified as "tumor-free". Patients whose TNM staging had to be modified as a consequence of the ISG results represent 7% of the entire patients population, and 34% of the previously classified "tumor-free" group.

The overall efficacy of ISG with F023C5F(ab')2 was 82.2%, resulting from the combination of a 79% sensitivity and a 96.7% specificity. The overall predictive value of a negative ISG result is therefore 50.6% (29.3% in CEA-seropositive patients, 65.1% in CEA-seronegative patients), while the predictive value of a positive ISG result is 99% (99.2% in CEA-seropositive, 94.2% in CEAseronegative patients).

As a part of the activities of this Research Contract for 1987, a study was undertaken to evaluate the efficacy of ISG as compared specifically with other noninvasive diagnostic techniques in detecting tumor lesions. To this purpose, clinical chart data of 200 patients selected from the total 542 patients submitted to ISG with F023C5F(ab')2 were analyzed comparing in particular the results of ISG with those of standard x-ray (either film x-ray and/or x-ray fluoroscopy), Transmission CT, and ultrasound examinations (either echography and/or echotomography).

Only patients with ascertained tumor lesions and in whom at least a standard x-ray examination had been performed in addition to ISG were selected for the present evaluation; furthermore, selection was limited to the two more homogeneous and numerically important types of disease, that is gastrointestinal cancer (131 patients) and lung cancer (69 patients). In all these patients the presence of tumor lesions had been ascertained by a combination of physical findings, surgical exploration, and follow-up.

As a consequence of the selection criteria just mentioned, there were obviously neither false positive nor true negative results at ISG, or at the other diagnostic techniques considered for the comparative evaluation.

The size of tumor lesions being considered as a critical factor for the usefulness of early detection of cancer recurrences, patients were further divided into two subgroups on the basis of tumor lesions size, respectively smaller or larger than 2 cm in diameter.

The data for evaluation of ISG efficacy as compared to standard x-ray

are reported in Table I. As expected, the highest degree of concordant results between the two techniques was found in the groups with tumor lesions larger than 2 cm: concordant results were obtained in 52.2% of cases in patients with gastrointestinal cancer (43.6% for lesions smaller than 2 cm) and in 56.7% of the patients with lung cancer (43.6% for lesions smaller than 2 cm). Concerning ISG sensitivity, data obtained in the selected groups were superimposable with the observations in the entire group of 542 patients included in the multicenter clinical trial, showing an overall sensitivity in the range of 80%. When these data are analyzed in relation to tumor size it is found that 76.9% and 83.3% of the tumor lesions, respectively smaller or larger than 2 cm, were detected by ISG in the lung cancer group, as compared with the sensitivity of x-ray in the same patients corresponding to 43.6% and 60% respectively.

As to the gastrointestinal cancer group, the results of ISG appeared to be particularly valuable for tumor lesions smaller than 2 cm (100% sensitivity as compared to 43.6% of x-ray), whereas ISG sensitivity in lesions larger than 2 cm was at the lowest degree observed in this series of patients, although still higher than for x-ray (69.6% versus 58.7%).

This findings may perhaps be explained by the fact that patients with more advanced stages of gastrointestinal cancer (lesions larger than 2 cm) are more likely to develop liver metastases; the performance of ISG regarding tumor localizations in the liver still remains quite poor, due to the high nonspecific accumulation in the liver of the monoclonal radiopharmaceutical, especially if labelled with indium 111. Comparison with CT (Table II) and ECHO (Table III) confirms the higher sensitivity of ISG.

The results of this study provide a good indication of the present status and diagnostic usefulness of ISG in tumor detection. On the basis of this preliminary prospective study, the next step will be the comparison of the resulting radiation exposure with the diagnostic efficacy of ISG, in comparison with x-ray, ultrasonography (echography and echotomography) and computer tomography in patients with melanoma labelled with Tc-99m.

Table I - x-rays vs. ISG

	Lesions < 2 cm			Lesions > 2 cm		
	x-ray+	x-ray-	x-ray±	x-ray+	x-ray-	x-ray±
Lung Cancer						
ISG+	13	13	4 (76.9%)	15	3	7 (83.3%)
ISG-	4	2	0 (15.4%)	1	2	0 (10.0%)
ISG±	0	1	2 (7.7%)	2	0	0 (6.7%)
	(43.6%)	(41.0%)	(15.4%)	(60.0%)	(16.7%)	(23.3%)
Patients n.: 37				Patients n.: 30		
ISG sensitivity: 76.9%				ISG sensitivity: 83.3%		
x-ray sensitivity: 43.6%				x-rays sensitivity: 60%		
Concordant results: 43.6%				Concordant results: 56.7%		

Gastrointestinal Cancer

ISG+	17	15	7 (100%)	40	18	6 (69.6%)
ISG-	0	0	0 -	8	4	1 (14.1%)
ISG±	0	0	0 -	6	5	4 (16.3%)
	(43.6%)	(38.5%)	(17.9%)	(58.7%)	(29.4%)	(11.9%)

Patients n.: 39
ISG sensitivity: 100%
x-ray sensitivity: 43.6%
Concordant results: 48.7%

Patients n.: 64
ISG sensitivity: 69.6%
x-ray sensitivity: 58.7%
Concordant results: 75%

Legends:

Patients with x-ray: 200
(+): positive results for tumor localizations
(-): negative results (false negative)
(±): questionable results as to tumor localizations.

Percentages in brakets refer to the sums of figures in horizontal lines or in vertical columns with respect to the patients' population of each subgroup.

Table II - CT vs. ISG

Gastrointestinal Cancer

	Lesions < 2 cm			Lesions > 2 cm		
	CT+	CT-	CT±	CT+	CT-	CT±
ISG+	11	2	4	21	3	1 (65.8%)
ISG-	0	0	0	3	1	0 (10.5%)
ISG±	0	0	0	4	2	3 (23.7%)
				(73.7%)	(15.8%)	(10.5%)

Legends as in Table I.

CT study: patients 38

GI cancer patients with tumor lesions 2 cm.

ISG sensitivity: 73.7%
CT sensitivity: 65.8%

Table III - ECHO vs. ISG

Gastrointestinal Cancer

	Lesions < 2 cm			Lesions > 2 cm		
	US+	US-	US±	US+	US-	US±
ISG+	4	14	5 (100%)	22	4	4 (63.8%)
ISG-	0	0	0	2	2	1 (10.6%)
ISG±	0	0	0	7	2	3 (25.6%)
	(17.4%)	(60.9%)	(21.7%)	(66.0%)	(17.0%)	(17.0%)

Legends as in Table I.

US: Echography + Echotomography
Patients: 70 (patients with echography: 25; patients with echotomography 45)

GI cancer patients with tumor lesions < 2 cm:
- 17.4% concordant results
- 100% ISG sensitivity
- 17.4% US sensitivity

GI cancer patients with tumor lesions > 2 cm:
- 75% concordant results
- 63.8% ISG sensitivity
- 66.0% US sensitivity

IV. Objectives for the next reporting period:

1) To feed the kinetic tissue distribution data obtained so far in the CAMIRD III program, for actually performing the calculation of internal radiation dosimetry to the patients.

2) To perform a similar tissue distribution study for at least an additional monoclonal tracer (an anti-adenocarcinoma).

3) To start an enlarged clinical trial with 131-I-F023C5-F(ab') and with 111-In-F023C5-F(ab'_2) in patients affected by cancers characterized by a significant expression of CEA by the tumor cells.

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:

RADIATION PROTECTION PROGRAMME

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Contractor: Contract no : BI6-F-132-F

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.

Head(s) of project:

C. Maccia

Scientific staff:

M. Benedittini, F. Fagnani, C. Lefaure, C. Maccia

I. Objectives of the project:

The final objectives of the project will be 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. Two differents assessments will be carried out : the first one, in close cooperation with the NRPB (UK) will be focused on the evaluation of the "remainder organs" contribution to the effective collective dose equivalent ; the second one will be to study the total population exposure due to the CT examinations by using a common dosimetric methodology elaborated with the GSF (Germany), the NRPB (UK), the USL n°7 (I) and the CEPN (F).

II. Objectives for the reporting period:

a) To contribute to the establishment of quality criteria for diagnostic radiographic images.

b) To start dosimetric measurement on CT.

III. Progress achieved:

a) Meetings have been held with a number of scientits involved in the Radiation Protection Programme of the CEC regarding the definition of a list of quality criteria for radiographic images as well as the possibility of setting up a multinational pilot survey aiming at improving them in the daily routine work of some European radiological departments.

For the purpose of the survey, six x-ray examination types have been selected and a questionnaire asking for image criteria evaluation, radiographic parameters, patient related data and doses has been specially designed. Data on about 1,000 x-ray examinations performed in about 25 radiological departments are being collected.

b) Measurements of central axis free in air dose have been started on a CT scanner unit according to the basic protocol agreed with the other contractors (NRPB, USL n°7, GSF). This demonstrated the simplicity of the measurement technique (a jig containing 12 TLDs to be irradiated in a single slice) and will enable further measurements to be carried out on a larger scale. Although the great majority of the CT scanner operating in France are made by CGR (CE 10000 machine) some measurement will be also carried out on other CT models.

IV. Objectives for the next reporting period:

a) To handle data collected in the multinational pilot survey and to proceed to their statistical analysis.

b) To design a computer programme which will enable us to evaluate the collective effective dose equivalent associated to the CT examinations through the Monte Carlo dose coefficient provided by the NRPB.

V. Other research group(s) collaborating actively on this project:

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:

M. Benedittini, C. Maccia, C. Lefaure, F. Fagnani - Doses to patients from dental radiology in France - Health Physics, 1988 (to be published).

G. Contento, C. Maccia, M.R. Malisan, R. Padovani, P.C. Shrimpton, B.F. Wall - A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy - British Journal of Radiology, Vol. 61, n° 722, 1988, pp. 142-152.

C. Maccia, M. Benedittini, C. Lefaure, F. Fagnani - Doses to patient from diagnostic radiology in France. Health Physics, 1988 (to be published).

F. Fagnani, C. Le Galès, F. Héran, C. Lefaure, C. Raybaud - La planification des équipements médicaux au niveau régional : le cas du scanographe dans la région PACA. INSER U.240 report n° 40, Nov. 1986, Report to ORS-PACA.

C. Le Galès, C. Lefaure, F. Fagnani, Y. Obadia, F. Héran - La pratique de la tomodensitométrie en période de croissance rapide du parc. Journal de Radiologie, 1987 (to be published).

Title of the project no.:

Quality assurance in medical diagnostic radiology

Head(s) of project:

C. Maccia

Scientific staff:

M. Benedittini, F. Fagnani, C. Lefaure, C. Maccia

I. Objectives of the project:

a) Assessment of the image detector system and the interpretation equipment quality on the doses received by patient undergoing diagnostic x-ray examinations.

b) Evaluation of both radiological practice and dose reduction possibilities in the case of the newborn pelvis x-ray and the mammary gland examination.

c) Assessment of the Digital Subtraction Angiography and its relevance in reducing patient dose.

II. Objectives for the reporting period:

The main objectives of this part of the five years projects are the following :

-To evaluate the status of the radiological equipment currently used in mammography (distributed patient dose, film image quality, type of detectors) and to promote initiatives in the field of Quality Assurance.

III. Progress achieved:

Quality Assurance for Mammography

Methods

In 1987 the CEPN together with the SFPH (Société Française des Physiciens d'Hôpital) conducted a survey on 75 dedicated mammographic units installed in both public hospitals and private radiological offices all over in France. This enables us to collect data about radiological techniques likely to influence both breast dose and image quality (kVp setting, anti-scattering grid, magnification).

A questionnaire was sent to all radiological centers asking for about 100 physical parameters for each x-ray unit : geometry, x-ray tube, focal spot size, target material, phototimer, grid,kVp, exposure time, film-screen combinations..).

From the collected data, three categories of units were selected for both dose and image quality evaluation. For this latter, Mammographic Random Phantom (RMI) was used, whilst for the dosimetry, measurements of entrance skin dose, depth dose and exit dose were carried out on a lucite phantom simulating a compressed breast of 4 cm.

About 50 radiograms of the RMI phantom, taken under different technical conditions (kVp, mAs,...) were randomly shown to six experienced radiologists in the usual interpretating conditions. Credit concerning informations on image quality (size of the objects embedded in the phantom) was given only for correclty identified objects by three viewers or more.

Results

Of the 75 considered units, about 85 % are equiped with a Mo fixed or rotating anode (respectively 15 % and 70 %) and the remaining ones are equiped with a fixed or rotating W anode (respectively 6 % and 9 %).

Three out of four x-ray tubes have only one focus (either 0.3 mm or 0.6 mm), and if a second focus exists, its size is generally of 0.1 mm.

40 % of the installations do not allow to take a mammogram with a focus to film distance above 48 cm.

Only 60 % of the practitioners actually use phototimer, although 72 % of total number of units are equiped with.

Anticattering grid is installed on 47 % of the considered mammography systems and only one third of those latter makes possible magnification technique to be practised.

Neither direct film mammography nor Xerox plate is used in the whole sample of participating centers and 12 screen-film combinations, often differents from those suggested by manufacturers, have been found.

Estimates of kilovoltage for a 4 cm "average" compressed breast show large discrepancies and range from 19 kVp to 42 kVp with a mean value of 28 kVp, all target materials together.

Practitioner's modifications towards a "dense" breast or a "fatty" one remain one of the most important sources of disparity encountered in the survey. In diagnosing a "fatty" breast for instance, some radiologists tend to increase physical parameters namely : the kilovoltage (16 %), the exposure time (5%) and the phototimer sensitivity (6 %). Conversely, some others, tend to decrease the same parameters (9 %, 12 %, 12 % respectively), 15 % put no modifications and the remaining 25 % have no opinion. Regarding a "dense breast examination, almost all radiologists set the parameters up : the kilovoltage (32 %), the exposure time (19 %), both kilovoltage and milliampere-second product (28 %), 10 % keep the parameters unchanged and, finally, 11 % have no opinion.

Besides mass screening consideration, 3 projections per breast are, on average, used routinely during an examination.

Entrance skin dose values may range from 6.2 mGy to 18.3 mGy depending on the unit characteristics and on the selected parameters.

Figures of 12 % and 2 % of the entrance skin dose were found respectively for the glandular breast tissue dose and the exit dose.

All these results, together with some other clinical aspects (examination technique as a function of the breast density for instance) call for a need of harmonizing all parameters which may influence either patient dose as well as quality of diagnosis.

IV. Objectives for the next reporting period:

A quality control programme for mammography will be defined in order to ascertain reliability of kilovoltage currently used. This certainly will allow to restrict discrepancies encountered in the survey with a consequental real effect of reducing dose while improving image quality.

Dose comparison between examinations performed on conventional mammographic units and on digitalized ones will be continued in cooperation with the Christie Hospital in Manchester.

V. Other research group(s) collaborating actively on this project:

B. M. Moores - Christie Hospital in Manchester.

VI. Publications:

Lefaure C., Fagnani F., Héran F., Benedittini M. - Digital substraction angiography in France. in : Proceedings of an International Symposium on Quality Assurance in Health Care, Paris, November 1986, pp. 56-59.

Lefaure C., Weill C., Fagnani F., Benedittini M., Friga G. - Aspects socio-économiques de la diffusion de l'angiographie numérisée en France. in : L'angiographie numérisée, Frija Ed., Editions Ellipse, 1987, pp. 363-369.

Castellano S., Maccia C. - L'assurance de qualité en radiodiagnostic : le cas de la mammographie. Rapport CEPN n° 120 - Juin 1987.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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.J.Glas, Dr.med.K.Drews, Dipl.Psych.M.M.Kohn, cand.med.Pehe, cand.med.Weisbach

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 survey was started 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. Similar data will be subsequently taken at other roentgen diagnostic centers. The acquired knowledge of the actual conditions under which roentgen examinations in children are performed at the different centers 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 February, these measurements will have been made at more than 30 study centers. Although all of these centers are under the supervision of a qualified and experienced paediatric radiologist, preliminary analysis of the data already shows that not only are there major differences between and within the individual centers, but also allows 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.

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 will follow this first series.

V. Other research groups(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

1987

Contractor: Contract no BI6-F-133-D

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

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

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Title of the research contract:

Analysis of exposure in radiology

List of projects:

1. Analysis of exposure in radiology

Title of the project no.: B16 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
Dr. G. Williams
Dipl. Phys. W. Panzer
Dr. N. Petoussi

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:

- Complete the work on the pediatric phantoms now available and start work on new whole body CT data for smaller and larger children.
- Complete the work on the available Alderson phantom.
- Continue the analysis of organ doses in radiotherapy procedures.
- Obtain CT whole body data for adults and construct phantom files.
- Continuation of the CT field studies.

III. Progress achieved.

The Monte Carlo computations of organ doses in radiology were continued; emphasis was given to pediatric radiology. For this, photon transport codes were applied to anthropomorphic realistic phantoms which were previously constructed from computer tomographic (CT) data of a baby (eight weeks old) and of a child (seven years old).

In this work, which is performed in cooperation with the Children's Hospital of the University of Munich, emphasis is given on estimating the amount of bone marrow in the skeleton and consequently on the dose delivered to the bone marrow. The amount of red bone marrow in each skeleton volume element (voxel) was assessed from the CT number of the corresponding picture element as the skeleton voxels have greatly varying densities representing different mixtures of hard bone and bone marrow. Thus, the proportion of bone marrow in a voxel was determined from the grey value of this voxel by interpolating between the grey value of bone marrow and the grey value of hard bone. Although it was not possible by this method to model the complicated trabecular bone structure exactly, the spatial distribution of the bone marrow in the whole skeleton was well approached.

Organ doses resulting from leukemia treatment by whole body irradiation with therapy Co 60 gamma rays were calculated for a child whose own CT data were used to construct a mathematical phantom. The irradiation conditions were exactly simulated, e.g. by using shielding blocks of different transmission, a personalised blocking lung filter, various projections and field sizes, etc.. While a dose of 12 Gy at middle line was intended by the physicians to be delivered during the treatment, the calculations of the bone marrow doses in the different bones were found to vary from 8.9 to 13.5 Gy.

For diagnostic radiology, influences of field size and tube voltage on organ doses were calculated, for most common X ray examinations in pediatric radiology; possibilities of reducing these doses were considered, e.g. by aligning the field carefully and as narrow as possible without losing valuable information or by choosing the highest of a range of high voltages rendering the contrast desired.

Computer tomographic data files processed by suitable software were used to construct three dimensional images of bone structures and organ surfaces. These images have a high resolution, i.e. 1.54 mm in width and depth respectively and 8 mm in height, and can be used for diagnostic or other medical purposes such as reconstructive or cancer surgery.

The Monte Carlo code which simulates photon transport and pursues the secondary electrons was considerably extended by introducing the possibility of calculating the geometrical dose distribution in arbitrary volumes of interest. These dose distributions can now be presented as three dimensional images.

An additional improvement of the Monte Carlo code was achieved by leaving the restriction to rectangular fields. This is important for dose calculations in radiotherapy since there often non-rectangular fields are applied. Examples are the blocking lung filters, used with the whole body irradiation for leukemia treatment, which are formed according to X-ray pictures of the patient's lungs or, in the case of irradiation of a well delimited tumor, the possible reduction of the irradiated field to the tumor's shape in "beam's eye view".

A whole body CT scan of an Alderson Rando phantom consisting of 122 8 mm slices was performed and data files were prepared for 3-dimensional reconstruction and Monte Carlo calculations.

The field study for the evaluation of dose values occuring in CT-examinations in Germany has been completed covering 122 facilities in hospitals and medical practices. The results can be concluded as follows:

- All the institutions were equiped with rotate only CT machines which were exclusively used in the 360° scan mode in contiguous slices; 9 participants reported overlapping slices in spine examinations.

For the number of slices per examination in the various body regions the following figures were found (mean number of slices ± standard deviation, range of number of slices):

Skull	20.3 ± 8.1	5 - 44
Thorax	27.4 ± 8.3	10 - 56
Abdomen	36.3 ± 14	10 60
Pelvis	24.4 ± 4.3	10 - 60
Spine	22.7 ± 7.3	10 - 40

For the examination of only single segments of the spine, slice numbers from 4 to 11 were reported.

- A certain preference of 8 mm slices in examination of thorax, abdomen and pelvis could be observed as well as for 4 mm slices for spine and the skull basis. However, all possible slice thicknesses were used for the different examinations in practice.

The lengths of the scanned body regions determined by the product of slice thickness and number of slices per exmination (as reported by the participants) showed a wide variation (mean length ± standard deviation, range, all figures are given in mm):

Skull	123 ± 49	20 - 264
Thorax	213 ± 58	100 300
Abdomen	262 ± 96	50 380
Pelvis	204 ± 68	50 400
Spine	73 ± 19	40 120

This large spread of a parameter most decisive for estimating the dose to the patient will complicate the preparation and presentation of data for this purpose.

- Beam profile analysis could be performed for 2 mm slices and 4 mm slices revealing an overlapping of contiguous slices due to the special construction of the collimating systems and/or its misadjustment. This effect causes a dose enhancement which has to be considered when performing patient dose estimations on the basis of single scan dose measurements free in air. The findings in the field study are given below (mean dose enhancement factor ± standard deviation, range):

2 mm slices	1.49 ± 0.35	1.01 - 2.26
4 mm slices	1.19 ± 0.32	0.88 - 2.22

- Dose values even for the same exposure conditions (tube voltage, filtration, distance) and normalized to the same product of the tube current and exposure time showed a surprising variation. This indicates the need for dose measurements when a realistic estimation of individual patient doses, e.g. embryo dose, becomes necessary. The use of dose values from output data collections referring to nominal exposure conditions would lead to unrealistic results, for part of the facilities.

IV. Objectives for the next 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 physically assessed doses.

  Continuation of construction of mathematical phantom files 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.

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

VI. Publications:

Petoussi, N., Zankl, M., Williams, G., Veit, R., Drexler, G.: The calculation of dose from photon exposures using reference human phantoms and Monte Carlo methods. Part V: Organ doses from radiotherapy for cervical cancer. GSF Bericht 5/87 (1987)

Williams, G., Veit, R., Schneider, K., Zankl, M., Wiechell, R., Petousssi, N., Fendel, H., Drexler, G.: The construction of 3D whole body images from CT data und the use of image processing methods to produce files for Monte Carlo dose calculations. In: Computer Assisted Radiology (Eds.: H.U. Lemke et al.). Springer Verlag, Berlin, pp. 148 152 (1987)

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no B16-F-134-IPL

Federated Dublin
Voluntary Hospitals
P.O.BOX 795
IRL Dublin 8

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

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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 reduction in radiological image intensifier - TV systems as an adjunct to quality control and optimization of exposure.

Title of the project no.: 1 Specification and Study of Uniformity and Signal to Noise Ratio in Image Intensifier/TV Systems.

Head(s) of project: J. F. Malone.

Scientific staff: P. Cooney,
K. Maher,
M.K. O'Connor.

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.

II. Objectives for the reporting period:

(a) The contribution of individual components in the imaging chain to overall non-uniformity will be looked at in more detail to ensure saturation or similar effects are not biasing results.

(b) The influence of the resolution of the matrix, into which images are acquired, on uniformity will be examined.

(c) Contact with other relevant bodies/groups will be further developed and the possibility of devising a protocol/alternative specifications will be further explored.

(d) Dosemetric studies will be continued.

(e) Initial studies on the noise level in images and subtracted images will be undertaken with particular reference to the influence of dose/dose rate and to the electronic contribution.

(f) The contribution of the individual components in the imaging chain to (e) will be evaluated.

III Progress achieved

Substantial progress was achieved under headings a , c and while progress under headings d , e and f was less than anticipated, primarily due to staff changes. Discussions in respect of the possibility of developing a protocol for the area have been continued with other contractors, professional associations and statutory bodies.

The influence of the resolution of the pixel matrix, into which images were acquired, on system uniformity measurements has been examined. Image resolution was reduced using a software method which was essentially a pixel averaging technique. "Improvement" in the measured system uniformity by factors as large as 0.33 were observed with smaller matrix size. This was expected due to smaller deviation between the pixel values as they were averaged over larger areas.

Methods have been developed to measure the uniformity of the individual system components of the imaging chain x-ray beam, image intensifier and T.V. camera . X-ray beam and image intensifier II measurements were performed in two planes:

(i) Perpendicular to the anode-cathode axis of the x-ray tube central horizontal plane as observed on the TV monitor .

(ii) Parallel with the anode-cathode axis of the x-ray tube central vertical plane as observed on the TV monitor .

Regular intervals of measurement were selected using an orthopaedic rule inserted into the beam. Given that measurements were only performed over two planes rather than over the entire field of view, a revised uniformity definition was required. Hence results were expressed as "horizontal band" uniformity and "vertical band" uniformity. The x-ray beam evaluation was performed using a dose meter (MDH, 2 and a cc ion chamber. II evaluation was performed by measuring its light output using a directional light meter Hagner Universal Photometer .

TABLE 1: Uniformity of Horizontal Vertical Band in X Ray Beam II Systems.

SYSTEM	BEAM UNIFORMITY		II + BEAM UNIFORMITY	
	Vertical	Horizontal	Vertical	Horizontal
1	± 15.0	± 7.6	± 3 .	± 21.3
2	± 9.0	± 5.0	± 25.	± 29.1
3	± 10.0	± 10.7%	± 20.	± 22.8
4	± 7.6%	± 6.1%	+ . .	± 21.1
5	± 16.0	± 5..	± 2.4	± 25.6%
6	± 16.6	± 6.2%	± 27.	± 25.2
7	± 17.0	± 13. %	+ 17.	+ 12.6
8	+ 1.8%	± 7. %	-	-
9	± 16.3%	+ . .	± . .1	± 1. .
10	+ 1.6%	± 9.7	± 23.4	+ 18.3

Independent stimulation of the II using a uniform flood source with an isotope of suitable energy proved impractional due to the high level of activity required. Thus the x-ray tube was used as the source for II uniformity analysis. TV camera uniformity analysis was performed by imaging a uniform light source with the camera removed from the II. Analysis was performed in the same way as for system uniformity, over both the usefull field of view (UFOV) and the central field of view (CFOV).

Component uniformity results are presented in Table 1 and 2, for ten conventional x-ray II-TV systems in use in a number of hospitals. Substantial variations between the systems can be seen for each of the measurements. For the x-ray beam, vertical band uniformity was greater than the horizontal band in all cases due to the influence of heel effect. Horizontal band uniformity was largely dependent on the oblique transmittion of the beam. The influence of heel effect is also seen in the vertical band uniformity of the II. Thus horizontal band uniformity gives a better indication of the "true" II uniformity. Improvement factors as high as 0.50 were observed for horizontal band uniformity with the introduction of II field size magnification. Thus the influence of edge effects such as the curved input surface of the II, oblique transmittion, heel effect and vignetting, was apparent.

The uniformity of the TV camera on its own, when viewing a uniform light source is presented in Table 2. Some systems were not examined as the camera could not safely be removed. An improvement in the uniformity when the CFOV only is selected is also noted here (Table 2). This improvement was due to a decrease in the maximum pixel value from the edge of the field of view towards the centre, thus indicating the effect of vignetting within the coupling optics of the camera.

TABLE 2: Uniformity of TV Image from Camera exposed to Uniform Light Source

SYSTEM	TV CAMERA UNIFORMITY	
	UFOV	CFOV
1	± 13.4%	± 8.1%
2	± 56.7%	± 48.3%
3	-	-
4	-	-
5	± 18.4%	± 13.8%
6	± 22.9%	± 14.5%
7	± 11.1%	± 7.3%
8	-	-
9	-	-
10	-	-

IV. Objectives for the next reporting period: (a Both system and c mponent 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 the component uniformities contribute to the total system uniformity will be made. 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 rati in images and subtracted images will be initiated with particular emphasis on the electronic contribution of the TV camera circuitory and the influence of dose/dose rate.
(g) Contact will be continued with other Research Group s involved in this area.

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

VI. Publications: IN SCIENTIFIC JOURNALS MONOGRAPHS:

Maher, K.P., O'Connor, M.K. and Malone, J.F., 1987. Experimental examination of videodensitometry f large opacifications in digital subtraction angiograhy. Phys. Med. Biol., 32, 1273 - 1282.

Maher, K.P., Malone, J.F., Hurley, G.D. and McInerney, D.P., 1987. Evaluation of the processing functions of a D.S.A. Image rocessor British Journal of Radiology, 61, 62 - 68.

Malone, J.F., Maher, K.P. and O'Connor, M.K., 1988. Quantitative Aspects of Digital Fluorography. Hospital Physicists' Association Conference Proceedings (in press).

SHORT COMMUNICATIONS, THESES, INTERNAL REPORTS:

Cooney, P., 1987. Uniformity Analysis in X-Ray Image Intensifier-TV Systems using a Digital Image Processor. M.Sc. dissertation, Department of Clinical Medicine, University of Dublin, Trinity College.

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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)]

Dr. A. F. McKinlay
Physics Department
NRPB
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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. Analysis of somatic doses and risks from routine X-ray procedures in British hospitals.
2. Assessment of the contribution of computed tomography to the collective dose from diagnostic radiology in Britain.

Title of the project no.: 1

Analysis of somatic doses and risks from routine x-ray procedures in British hospitals.

Head(s) of project:

Mr B F Wall

Scientific staff:

Dr P C Shrimpton

I. Objectives of the project:

To use information on the organ doses and energy imparted to patients to estimate the somatic risks from common types of x-ray examination.

To evaluate the collective doses and risks from diagnostic radiology in Britain for comparison with other sources of radiation and with other European countries and to identify those techniques that are responsible for high patient doses.

II. Objectives for the reporting period:

1. To report on collective doses and risks from routine x-ray examinations in Britain.

2. To report on a comparison of radiology practice and the resulting patient doses in France, Italy and Britain.

3. To investigate the reasons for the wide distributions in patient doses observed for nominally the same type of examination in our recent somatic dose survey.

III. Progress achieved:

Project completed in 1986.

No further progress apart from publications listed.

IV. Objectives for the next reporting period:

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

VI. Publications:

A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy. Contento G, Malison M R, Padovani R, Maccia C, Wall B F and Shrimpton P C, Brit. J. Radiol. 1988.

Patient dosimetry techniques in diagnostic radiology. Wall B F, Harrison R M and Spiers F W. IPSM Report No 53. (The Institute of Physical Sciences in Medicine, York).

Title of the project no: 2

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:

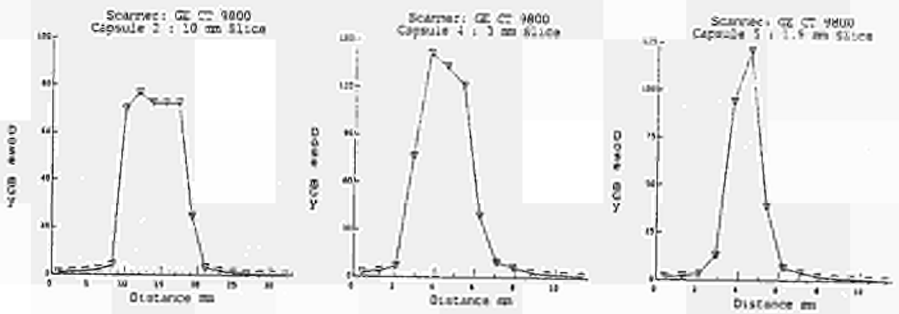
1. To commence a national survey of the level of provision of computed tomography services in the UK and the associated patient doses.

2. To develop further Monte Carlo calculations, simulating a wider range of CT procedures, to evaluate the relationship between organ doses in patients and the doses measured on the central axis of CT scanners.

III. Progress achieved:

Our proposed national survey of computed tomography (CT) practice and patient doses was introduced to an Institute of Physical Sciences in Medicine (IPSM) meeting in Birmingham in March 1987, where a number of hospital physicists agreed to co-operate in trials of our survey and dosimetry methods. These trials have now been successfully completed at 15 CT scanners in the UK. Dosemeters, supporting jigs and questionnaires were sent by post and were used by local physicists and radiographers to perform measurements and record the necessary data. With only minor modifications the same system will be used to complete the national survey next year.

The dosimetry method has evolved from correspondence and collaborative meetings in March and November with the other CEC contractors engaged in CT surveys. It consists of making direct measurements of the free-in-air axial dose profile for a single slice at the centre of rotation of each CT scanner, for each commonly used set of scanning parameters. TLDs supported in a suitable jig are provided for these measurements. Software has been developed at NRPB for graphically displaying these dose profiles to provide an immediate comparison of the collimator performance and dose requirements of different scanners as shown in the example below.



CT scans of a mathematical phantom representing an average adult patient have been simulated using Monte Carlo techniques, and factors relating mean organ doses to the measured axial dose profiles have been calculated. X-ray spectra and scan beam geometries appropriate for a

Siemans scanner have been used so far. Further sets of calculations are being performed for the other makes of scanner in use in the UK. About 100 contiguous 10 mm thick slices from the top of the head to the bottom of the trunk are simulated in the calculations and methods have been developed for selecting appropriate sets of slices to model any type of CT examination. The effective dose equivalent can be estimated with a statistical uncertainty of less than $\pm$ 2% for typical CT examinations.

Data on scanning techniques and scanner workload are requested in the questionnaire that is being sent to most of the CT facilities in the UK. Additional data have been obtained from the computerised patient logging systems that are incorporated in some makes of scanner. Together with information from the Department of Health, a reliable estimate of the national total of CT procedures per year will be made. The collective effective dose equivalent from this major source of medical x-rays will be determined.

IV. Objectives for the next 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.

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

1987

Contractor: Contract no B16-F-140-UK

Victoria University
of Manchester
Oxford Road
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Head(s) of research team(s) [name(s) and address(es)]

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Telephone number: 061/445.8123

Title of the research contract:

An assessment of the effect of digital technology on quality assurance procedures, including the evaluation of risks, in X-ray medical diagnosis.

List of projects.

1. An assessment of the effect of digital technology on quality assurance procedures, including the evaluation of risks, in medical X-ray diagnosis.

Title of the project no.: B16 - F - 140 - UK

An assessment of the effect of digital technology on quality assurance procedures, including the evaluation of risks in medical X-ray diagnosis

Head(s) of project:

B M Moores

Scientific staff:

T Dovas
P Rowlands
J R H Yarwood

I. Objectives of the project:

To establish baseline performance standards for digital radiology and to employ the numerical data generated by these systems to assess risks to patients undergoing examinations. Also, to assess the impact of digital technology on quality assurance procedures and radiation protection measures.

II. Objectives for the reporting period:

a) To continue development of measurement techniques for assessing the performance of digital X-ray imaging systems which are applicable to routine quality control procedures.

b) To assess the effect of digitization processes on image quality

c) To evaluate the effects of data processing on image quality, in particular their effect on visual detection of simulated lesions

d) To commence development of contour mapping techniques for outlining critical organs.

III. Progress achieved:

1. Methodology

A digital ionography system has been employed as a source of digital radiographic data. Images of test phantoms are produced under a variety of different radiographic conditions. The resultant images are digitized under a variety of different conditions. For instance the sampling frequency is varied by a factor of 2X. The effect of these changes on image quality is being studied.

A digital chest image is used to assess the effect of data processing on image quality through the use of visual detection tests employing simulated lesions. The processing operations include grey- scale transformations, histogram operations, edge-enhancement and smoothing functions. The visualisation of the simulated lesions has been evaluated by a number of observers both pre and post processing.

Contour mapping operations are under development using a rib edge detection process as a first step.

2. Results and Discussion

A digital chest image with superimposed simulated lesions is shown in figure 1. The position of the lesions was chosen so that they were superimposed on a variety of different types of structural detail. The amplitude assigned to each lesion so that it was just comfortably visible depended strongly on the immediate background. The assigned amplitudes were as follows:

Lesion No.	Background	Amplitude
1	Steep edge	95
2	Intersection of 2 edges	120
3	Steep circular edge	65
4	Course structural detail	85
5	Random noise	28
6	Random noise	26
7	Fine structural detail	65

These results clearly indicate the effect of structural noise on cotrast detectability. Although 12 different types of processing were employed they all led to the same general conclusion. Where a lesion is on a reasonably even background it is easy to enhance and virtually any processing will produce an improvement. Conversely if a lesion is against a structured background such as a rib then there appears to be very little which can be done to enhance it. Perhaps subtraction of known structures from an image might be useful.

Software packages for performing physical measurements of noise and resolution from images of teset phantoms have been implemented. Measure-

ments from a bar pattern resolution phantom can be used to calculate the contrast transfer function. Figure 2 shows the computed transfer function for the digital ionography system when used with X-ray beams of different quality. Routine quality assurance measurements can be performed directly on digital X-ray imaging systems and these will provide an indication as to the performance of these systems under different radiographic and/or operational conditions.

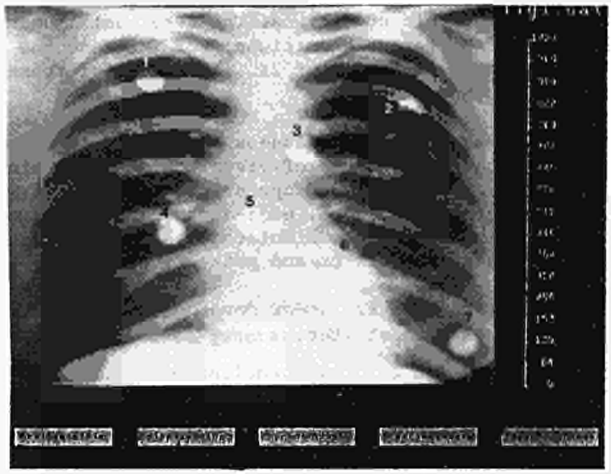

Figure 1 Digital chest image with superimposed lesions

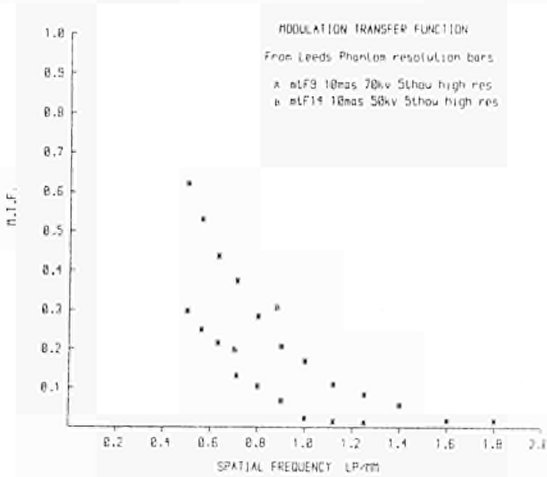

Figure 2 Computed modulation transfer functions

IV. Objectives for the next reporting period:

1. To further develop and implement routine quality assurance procedures for digital X-ray imaging systems.
2. To implement Receiver Operating Characteristic ROC analysis as an aid to evaluating the performance of data processing routines.
3. To implement organ contour mapping algorithms for assessment of risk estimates in diagnostic radiology.

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

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92260 Fontenay aux Roses
France

Dr J F Malone
Federated Dublin Hospitals
PO Box 795
Dublin 8

VI. Publications:

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no BI6-F-136-I

Unità Sanitaria Locale
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I-33100 Udine

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

Dr. R. Padovani
Serv.di Fisica Sanit.dell'
Osp.S.M.della Misericordia
Via Pieri
I-33100 Udine

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.: 1

Head(s) of project: Dr. Renato Padovani
Servizio di Fisica Sanitaria
Ospedale S. Maria della Misericordia
Udine

Scientific staff: Gilberto Contento
Mario Fabretto
Maria Rosa Malisan

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 evaluate the main factors affecting patient exposure in the examinations of skull, spine and breast according to the protocols established by the CEC Group on quality criteria for radiodiagnostic images.
2. To conduct a national survey on CT procedures.

III. Progress achieved:

1. A dosimetric survey has been conducted in two of the ma or radiology departments of the region concerning the six examinati ns for which the CEC Study Group on Quality Criteria for Diagnostic Radi graphic Images proposed a list of quality criteria and a questi nnaire. Doses have been measured by means of the TLD system developed for the previous regional survey. Data concerning chest, skull, pelvis, lumbar spine, urinary tract and breast examinations have been collected for a total of 120 patients.

2. A questionnaire has been prepared for the national statistical survey that is scheduled March, 1988. An analysis of frequencies of CT examinations carried out in 1983 has evidenced the 10 procedures that are most frequently performed; they are: head, chest, abdomen, cervical spine, lumbar spine, pelvis, petrous bone, hypophysis, orbits, skeletal segments. The type of procedure defines the anatomical compartment involved in the irradiation, the technical parameters define the beam characteristics that have to be simulated in Monte Carlo computations, the examination frequencies together with the measurement of free air doses at the axis of rotation allow to assess the collective doses due to each type of procedure. Therefore the following data will be requested for each procedure: kilovoltage, filtration, mAs, number of pulses, pulse length, number of slices, slice thickness, couch increment, scan angle, number of examinations per week. A scout-view typical for each is procedures is requested as a supplemental information to help identify the anatomical landmarks of the irradiated part of the body to compute organ doses.

The scope of the research and the questionnaire have been illustrated on the official Journal of the Italian Radiologist Society (SIRMN) to promote the survey among the users of the more than 250 CT installations in Italy.

IV. Objectives for the next reporting period:

1. Patient doses will be compared with those assessed in the 1983 survey in the same radiological departments. The comparison will evidence the trend of patient doses and the technical parameters responsible for such variations.
2. The analysis of the data collected on CT installations will be completed. A representative sample of CT installations including all types of CT scanners operating in Italy will be selected where dose in free air at the rotation axis will be measured. The assumption that, for each of the 10 selected procedures, the typical examination can satisfactorily represent all the examinations needs substantiation for what concerns dose estimation and will be tested.

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

Carlo Maccia, CEPN, Paris, France
Barry Wall and Paul Shrimpton, NRPB, Chilton, UK
Valerio Barbina, CRAD, Udine, Italy
Ludovico Dalla Palma, Gino Gozzi and Roberto Pozzi Mucelli,
Istituto di Radiologia, Università di Trieste, Trieste, Italy

VI. Publications:

R Padovani, G Contento, M Fabretto, M R Malisan, V Barbina and G Gozzi, 'Patient doses and risks from diagnostic radiology in North-East Italy', Br. J. Rad., 60, 155-65, 1987

G Contento, M R Malisan, R Padovani, C Maccia, B Wall and P Shrimpton, 'A comparison of diagnostic radiology practice and patient exposure in Britain, France and Italy', Br. J. Rad., 1988

C Maccia, B Wall and R Padovani
'Quality assurance in diagnostic radiology: patient exposure and radiological procedure comparison in Britain, France and Italy', VIth European Congress of Radiology, Lisboa, 1987

RADIATION PROTECTION PROGRAMME

Progress Report

1987

Contractor: Contract no BI6-F-137-D

Universität Erlangen-Nürnberg
Schlossplatz 4
D-8520 Erlangen

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

Prof. Dr. H. Pauly
Institut für Radiologie
Univ. Erlangen-Nürnberg
Krunkenhausstrasse 12
D-8520 Erlangen

Dr. Th. Schmidt
Radiologisches Zentrum
Klinikum der Stadt Nürnberg
Flurstrasse 17
D-8500 Nürnberg

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.:

BI-6-0137-D (B)

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
Dr. rer.nat. G. Böhnlein
Dr. rer.nat. M. Wucherer
cand. med. H. Erle

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:

- system dose for different recently devellopped film-screen-systems
- quality control of dental X-ray units

III. Progress achieved:

1. Determination of system doses for a new film-screen system

Even today the major contribution to civil radiation exposure is due to X-ray diagnostics.

The radiation exposure of the patient essentially depends upon the sensitivity of the imaging system, i. e. the system dose. Considering X-ray photographs the system dose is given by the respective film-screen combination. Thus, the efforts of the industry has always been an enhancement of both film sensitivity and effect of intensifying screens, without lack of resolution.

Since the early days of X-ray-technique the sensitivity of film-screen systems has been increased by a factor of 1000. Today there are more than 200 film screen systems.

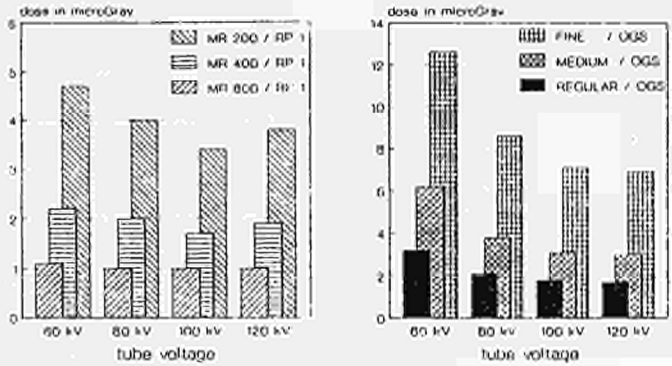
The dependence of system doses on tube voltage has been determined for the new Agfa Gevaert film screen systems (CURIX) at a net optical density of 1 (figure I).

All of the film screen combinations showed a decreasing system dose for tube voltages between 60 kV and 120 kV.

Compared to current rare earth intensifying screens, the systems 1 - 3 (see figure I) showed a reduction of system dose by a factor of 2. Thus, the radiation exposure or the somatic dose index has been reduced by a factor of 4 compared to conventional $CaWo_4$-screen systems.

figure I Film dose in µGy at net optical density 1 for the film screen system Agfa Gevaert CURIX (CURIX RP 1/CURIX ORTHO GS)

tube filtration: 2 mm Aluminium scattering body: water phantom DIN6815/2
field size: 25 x 25 cm² focus film distance: 100 - 140 cm variable
tube current: 50 - 500 mA dose read out device: PTW-DALi standardized
variable



2. Quality control of dental X-ray units

The number of X-ray exposures and the radiation exposure give reason for a critical investigation of dental X-ray examinations too, although the field size applied is considerably small. Apart from radiation exposure the radiation quality, the field size, the conditions of film development and the image quality (avoidance of repeated examinations) play an important role. Some remarks about radiaiton exposure at different dental X-rays can be found in literature, other parameters which determine the radiation exposure have not been investigated. Therefore our intention was, to determine field size, field homogenity, radiation exposure per examination and radiation quality for different dental X-ray units (table I).

table I Quality test at different dental X-ray units (random sample)

parameters investigated	mean deviation from nominal value in percent	standard deviation
tube voltage	- 8	± 2
exposure	- 29	± 38
field size	4,4	± 4,6
dose	---	± 33

IV. Objectives for the next reporting period:

1. Continuation with investigations considering the expediency of quality controls
2. Investigation of long term constancy of dental X-ray units
3. Dose reduction of lung examinations

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

VI. Publications:

Rehm H.-J., Th. Schmidt und K. Ewen:
Ermittlung der Strahlenqualität
in: Praxis der Qualitätskontrolle in der Röntgendiagnostik
(Hrsg.: H.-St. Stender, F.-E. Stieve)
Gustav Fischer Verlag, Stuttgart, New York 1986, 167 - 181

Erle H.:
Qualitätskontrolle an Dental-Röntgenanlagen; Dissertation 1988

RADIATION PROTECTION PROGRAMME

Progress Report

1987

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 MEDICAL DIAGNOSTIC RADIOLOGY

Head(s) of project:

Prof. E. Vaño and Prof. L. Gonzalez

Scientific staff:

Prof. A. Calzado, Prof. V. Delgado, Prof. P. Moran, P. Ortiz and M. Marin.

I. Objectives of the project:

-Risk analysis from medical Diagnostic Radiology to operation staff and population by means of area and personal dosimetry, and its correlation with equipment characteristics, workload and training of operation personnel.

-Risk analysis to patients by means of direct dose measurements and numerical estimations

-Design and starting of a pilot programme of quality assurance at hospital level.

-Establishing the bases for a future national programme of population dose estimation as a consequence of Diagnostic Radiology.

II. Objectives for the reporting period:

-Risk analysis in three rooms of similar characteristics (chest, digestive and special fluoroscopic) in the four Hospital Centres where the project has been carried out, through:

a) Area and personal dosimetry, in relation to workload and facilities.

b) Analysis of conditions under which examinations are performed in rooms controlled and patient dose estimations.

-Analysis of previous parameters for a national population dose survey programme.

III. Progress achieved:

METHODOLOGY

A risk analysis from area, personnel and patient dosimetry was carried out in the Diagnostic Radiology rooms of four large Hospital Centres of Madrid, which perform over 500,000 examinations per year. Chest, digestive, special fluoroscopic, angiography, emergency, urology, mammography and traumatology rooms, among others, have been controlled.

Over 2 500 area dosimetric measurements (using TL dosemeters), were carried out in rooms controlled monthly over a period of time ranging between 2 and 8 months, depending on the number of examinations performed. Occupational doses have simultaneously been controlled, including hand and lens measurements when necessary, relating them to the type and number of examinations carried out in each room. The data gathered from each examination for the different patients, comprised: exposure and film number (useful and rejected), kVp and mAs, screening time, age and sex. At this level, data from about 60 000 patients have been used in order to obtain the mean technical conditions of the examinations, as well as patient age histograms.

Patient organ doses have been derived from free-in-air ion chamber measurements, or through direct measurements in REMAB and RANDO phantoms (Alderson, USA). In the former, the NRPB protocol and conversion coefficients have been used for kVp and filtration values under mean and extreme operating conditions. In the latter, 30 to 50 individually calibrated LiF TLD chips have been used under average examination techniques. Also and parallel to performing the "Quality criteria for diagnostic radiographic images" programme promoted by the CEC, entrance surface dose, accessible organ dose (testes, breast and others) and dose x area product measurements were performed for each individual patient, using TLD-100 chips and Diamentor Chambers (PTW-Freiburg, FRG). A comparison between readings of our own dosemeters and those from the CEC programme is expected to be made.

The computerization process which will enable us to automatically handle (at least in part), the experimental data obtained, is currently in progress. The centres, rooms, operation staff, equipment features, area dosemeter positioning, examination types and characteristics, have been coded. A second phase interrelating area, personnel and patient dosimetry values, with currently coded parameters is intended for, leading towards detection of abnormal values and their possible cause (within a QA pilot programme).

Likewise, data from other Madrid area Centres and the rest of Spain obtained through the "Instituto Nacional de la Salud (INSALUD)" have been gathered and processed. Significant information on the state of Spanish Diagnostic Radiology, can be inferred from these data. This will lead us to planning a nationwide programme for assessing the risks involved in Diagnostic Radiology.

Using data provided by the National Institute of Statistics, the child expectancy for the Spanish population has been calculated, allowing us to estimate the Genetically Significant Dose (GSD), for some types of examination.

RESULTS

The following results can be pointed out:

a) A total of 78 rooms have been controlled, obtaining abnormal area dosimetry values (>1 mSv/month) in approximately 5% of them. Such values could involve possible risk situations for the staff and imply an incorrect structural barrier design. The workload values for these rooms have ranged from 200 (chest), to 900 mA.min/week (special fluoroscopy).

b) Intercomparing personnel dosimetry readings showed a reasonable agreement among the different lecture centres. Traumatology surgical rooms, catheterization, special fluoroscopy, angiography and conventional fluoroscopy rooms, have shown the highest values (up to 10 mSv/month). Hand and lens dose readings have reached up to 55 and 5 mSv/month respectively, on the staff of the aforementioned rooms.

c) Mean technical conditions for "complex" examinations, varied noticeably among centres. Average values of 13 and 20 exposures with screening times between 1 and 10 min for barium meal examinations were obtained. These values ranged from 8 to 14 exposures and 2 to 5 min of screening time for barium enema.

d) Organ doses were measured directly on anthropomorphic phantoms for some examinations (chest, digestive, conventional tomography, IVU, CT scans, etc.). In some complex examinations using the REMAB phantom, the contrast medium was introduced in the corresponding organ in order to better simulate real examination conditions. The following values of absorbed dose (in mGy) were obtained for a standard barium meal (15 exposures and 4 min screening time): entrance surface 35; thyroid 0.34; testes 0.25; ovaries 1.5; bone marrow 5.4; lung 2.6; breast 2.4; pancreas 17; liver 16; right kidney 11; left kidney 32; stomach 29; intestine 4.3.

e) Average values of 2.7 films/examination for hospital radiology, and 2.0 for outpatient and private practice in the Community of Madrid, have been inferred from the data obtained from the centres involved in the project and from other organisations. This represents a weighted average value of 2.24 films/examination.

f) Organ dose, effective dose equivalent, collective dose and GSD values for the previously referred to mean conditions, have also been estimated for the different types of examinations. The estimated collective dose values from the 490 exam./(1 000 inhab.year) for the whole of Spain and 580 for the Community of Madrid, have been 32 500 and 5 100 man.Sv, respectively (excluding routine labour and military controls and dental radiology). The GSD of chest examinations (150 exam./(1 000 inhab.year)) has been estimated to be 0.2 microSv, and 17 microSv for urinary tract examinations (15 exam./(1 000 inhab.year)), in the Community of Madrid.

IV. Objectives for the next reporting period:

-Risk analysis shall be completed performing area and personal dosimetry, in the remaining rooms of the four Hospital Centres involved in the project, and some other outpatient centres associated to them.

-Analysis of technical conditions and protocols of those examinations bearing the highest contribution to the effective dose equivalent.

-TLD and transmission ion chamber measurements on patients and phantoms, in order to obtain patient organ dose of the aforementioned examinations.

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

-Diagnostic Radiology Services of: San Carlos University, Primero de Octubre, G"mez Ulla Military and La Princesa Hospitals. Madrid.

-Radiation Protection Unit. Hospital de la Princesa. Madrid.

-Dosimetry Unit. Department of Health and Consumer Affairs. Madrid.

-Radiation Protection Unit. Hospital Primero de Octubre. Madrid.

-Energy Study Institute. CIEMAT. Madrid.

VI. Publications:

-Dosimetric evaluation in diagnostic radiology installations in Spain. L. Gonzalez, E. Vaño, A. Calzado, V. Delgado, P. Moran, P. Lopez and M. Bezares. Excerpta Medica International Congress Series (in press).

-Some indicative parameters of diagnostic radiology in Spain. First dose estimations. E. Vaño, L. Gonzalez, A. Calzado, P. Moran and V. Delgado. Data Source Report for UNSCEAR. September 1987.

-Influence of the X-ray generator and image system features on the patient exposure in chest examinations. E. Vaño, L. Gonzalez, P. Ortiz, P. Moran, V. Delgado, A. Calzado, P. Lopez. Proc. III International Symposium of Biomedical Engineering. Madrid. October 1987, pp 201-204 (in Spanish).

-Computerization of data from the "Optimization of protection in medical diagnostic radiology" project. E. Vaño, L. Gonzalez, M. Marin, A. Calzado, P. Moran y V. Delgado. Proc. III International Symposium of Biomedical Engineering. Madrid. October 1987, pp 175-176 (in Spanish).

IV

KOORDINIERUNGSTÄTIGKEIT

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 1987 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 1987

Study Group "Intercomparison programme on beta dosemeters"

Luxembourg (L), 22-23 January 1987
7 participants from 5 countries and the Commission

Principal subjects:
- Analysis of the results of the beta intercomparison programme
- Preparation of the fifth information seminar on beta dosimetry

Study Group "Improvement of practical countermeasures - preventive medication", Post-Chernobyl Action

Brussels (B), 27 January 1987
8 participants from 6 countries and the Commission

Principal subjects:
- Risks of administration of stable iodide
- Protocols and criteria for administration especially to pregnant women and children

Study Group "Evaluation of the reliability and meaningfulness of long-distance atmospheric transfer models", Post-Chernobyl Action

Brussels (B), 29 January 1987
7 participants from 5 countries and the Commission

Principal subjects:
Defining the following areas requiring additional research:
- Development of a module enabling to do back calculation from environmental data to the source term in a real time mode
- Correlation, hierarchisation and homogeneisation of the Chernobyl fall-out data
- Improvement of the trajectory model
- Dispersion over complex terrain

Study Group "Methods for assessing the radiological impact of accidents"

Brussels (B), 2 February 1987
25 participants from 10 countries and the Commission

Principal subject:
- The sixteen ongoing contracts and the two newly accepted research programmes were discussed

Study Group "Treatment and biological dosimetry of exposed persons", Post-Chernobyl Action

Brussels (B), 13 February 1987
9 participants from 5 countries and the Commission

Principal subjects:
- Development of an expert system to aid in decision making for treatment
- New approaches to enhance stem cell replication and differentiation
- Possibilities and limits of bone marrow transplantation after accidents
- Management strategies for the treatment of patients exposed in the LD50 - LD90 range

Study Group "Monitoring and surveillance in accident situations", Post-Chernobyl Action

Brussels (B), 16 February 1987
9 participants from 8 countries and the Commission

Principal subjects:
- Preparation of a working plan
- Specification of particular control and assessment of activity levels in foodstuffs and fodder

Study Group "Radiological aspects of nuclear accident scenarios", Post-Chernobyl Action

Brussels (B), 17 February 1987
13 participants from 6 countries and the Commission

Principal subjects:
Defining the following areas requiring additional research:
- Assessment of the cost effectiveness of countermeasures
- Real time assessment considering adaptations of atmospheric dispersion modelling and handling of radiological data

Study Group "Evaluation of data on the tranfert of Radionucleides in the food chain", Post-Chernobyl Action

Brussels (B), 18 February 1987
16 participants from 9 countries and the Commission

Principal subjects: Definition of the post-Chernobyl priorities
- Impact of the chemical speciation on the environmental tranfer
- Contamination of the natural ecosystems
- Transfer food-animal
- Contamination of impounded freshwater ecosystems
- Food processing
- Validation of transfer parameters

Study Group "Improvement of practical countermeasures - agricultural and aquatic environment", Post-Chernobyl Action

Brussels (B), 19 February 1987
9 participants from 6 countries and the Commission

Principal subjects: Definition of the post-Chernobyl priorities
- Setting up of scenario's
- Reduction of contamination near and distant the accident site

Study Group "Feasibility of studies on health effects due to the reactor accident at Chernobyl", Post-Chernobyl Action

Brussels (B), 25 February 1987
6 participants from 3 countries and the Commission

Principal subjects:
- Preparation of the research programme
- Workplan for the first six-month period of the project

Study Group "Improvement of practical countermeasures in the urban enviornment", Post-Chernobyl Action

Brussels (B), 26 February 1987
7 participants from 4 countries and the Commission

Principal subjects:
Defining areas requiring additional research:
- Further development of practical decontamination measures of urban structures
- Fundamental physico-chemical mechanism of retention on and diffusion in urban materials

Study Group "Underlying data for drived emergency reference levels", Post-Chernobyl Action

Brussels (B), 9 March 1987
7 participants from 4 countries and the Commission

Principal subjects:
- Metabolic dosimetric models
- Distribution and consumption of food after an accident
- Comparison of the Article 31 group approach with radioecological models
- Emergency management

Study Group "Optimization of radiological protection and occupational exposure"

Neuherberg (D), 12-13 March 1987
20 participants from 6 countries and the Commission

Principal subject:
- Assessment of the progress achieved over the years 1985 and 1986 to provide some input into the revision of ICRP publication n° 26

Study group "Quality criteria for diagnostic radiographic images"

Brussels (B), 16-17 March 1987
5 participants from 3 countries and the Commission

Principal subjects:
- Guidance notes for the implementation of a series of quality criteria
- Establishment of the test procedure

Study Group "Intercomparison programme on beta dosemeters"

Luxembourg (L), 2-3 April 1987
7 participants from 5 countries and the Commission

Principal subjects:
- Analysis of the results of the beta intercomarison programme
- Preparation of the fifth information seminar on beta dosimetry

Study Group "Radiation protection dosimetry"

Luxembourg (L), 28 April 1987
16 participants from 8 countries and the Commission

Principal subjects:
- Reports of activities in the field of radiation protection dosimetry
- Future work

Study Group "Applications of flow cytometry techniques in radiation biology and radiation protection research"

Brussels (B), 21 May 1987
12 participants from 7 countries and the Commission

Principal subjects:
- Presentation of recent developments in flow cytometry with emphasis on detection of abnormal chromosomes and aberrations
- Discussion of future perspectives and application in radiation protection research

Study group "Dynamic environmental cycling of tritium"

Mol (B), 21-22 May 1987
11 participants from 4 countries and the Commission

Principal subjects:
- HT deposition and conversion in soils
- HT deposition to plant surfaces
- Tritium tranfer to mammals
- Tritium in the aquatic environment

Study group "Quality assurance in medical diagnostic radiology"

Brussels (B), 17 June 1987
5 participants from 3 countries and the Commission

Principal subject:
- Frame and objectives of the Workshop on "Technical and Physical Parameters for Quality Assurance in Medical Diagnostic Radiology : Tolerances, Limiting Values and Appropriate Measuring Methods" Brussels, 23-25 February 1988.

Study group "Intercomparison programme on beta dosemeters"

Luxembourg (L), 18-19 June 1987
8 participants from 5 countries and the Commission

Principal subject:
- Preparation of the proceedings of the fifth information seminar on beta dosimetry held on 25-27 May 1987

Study Group "Practical countermeasures in the urban environment"

Leuven (B), 1 October 1987
7 participants from 4 countries and the Commission

Principal subjects:
- Co-ordination of the corresponding post Chernobyl action
- Report on the preliminary results obtained

Study Group "Methods for assessing the radiological impact of accidents" (MARIA)

Chilton (GB), 20-21 October, 1987
25 participants from 10 countries and the Commission

Principal subjects:
- Evaluation of the state of progress of the MARIA programme
- Possibilities of co-ordinating the development of the MARIA and CONDOR code

Study group "Quality assurance in diagnostic radiology"

Luxembourg (L), 21 October 1987
7 participants from 3 countries and the Commission

Principal subjects:
- Results of the preliminary study on the dosimetric instrumentation for quality assurance in diagnostic radiology
- Definition of an intercomparison programme on dosimetric instruments used in diagnostic radiology

Study group "Quality assurance in medical diagnostic radiology"

Chilton (GB), 11-12 November 1987
6 participants from 3 countries and the Commission

Principal subjects:
- Common protocol on dose assessment in computed tomography
- Outline for the Workshop on "Optimisation of Image Quality and Patient Exposure in Diagnostic Radiology."
  Oxford, 27-29 September 1988
- Priorities for 1990-1994 in the field of medical exposure

Study Group "Restricted intercomparison programme on environmental dosemeters"

Luxembourg (L), 2-3 December 1987
13 participants from 5 countries and the Commission

Principal subjects:
- Results of the Workshop on environmental dosemeters held in Braunschweig and Roskilde on 10-14 August 1987
- Preparation of the summary report
- Future work

Study Group "TLD environmental intercomparison"

Brussels (B), 15 December 1987
11 participants from 6 countries and the Commission

Principal subjects:
- Discussion of the preliminary results
- Preparation of a report on the results to be published in 1988

B

MEETINGS ORGANIZED OR CO-ORGANIZED BY

THE COMMISSION OF THE EUROPEAN COMMUNITIES IN 1987

Workshop on "Radiological Consequences of Chernobyl"

Brussels (B), 3-5 February 1987
80 participants from 16 countries and the Commission

Principal subjects:
- Evaluation of the radiological consequences of the accident
- Validation of the atmospheric transport models
- Definition of the still existing gaps in consequence assessment models

EULEP Task Group Meeting on "Molecular approach to radiation-induced osteosarcomas"

Sandjberg (DK), 6-8 February 1987
11 participants from 3 countries

Principal subjects:
- transformation in vitro of osteoblast-like cells by osteosarcoma virus
- osteosarcoma-like transformation in organ culture of cartilage
- effects of interferon on transformed cells in vitro
- analysis of murine leukaemia viruses causing skeletal disorders

EULEP Task Group Meeting on "Development of the CNS"

Freiburg (D), 12-13 February 1987
12 participants from 6 countries

Principal subjects:
- radiation damage to the fetal central nervous system
- comparison of X-rays and neutrons; effects with ^{131}I and ^{125}I
- biochemical studies in irradiated fetal brain
- effects on neuronal organisation in the developing brain
- cell migration in the cerebellum
- glial cell development

EULEP Annual Assembly

Reisensburg (D), 9-12 March 1987
70 participants from 7 countries and the Commission

Principal subjects:
- Presentation of EULEP activities during 1986
- Proposals for cooperative task-groups 1987
- Discussion of future trends in late effect studies in radiation protection research
  (Two workshops and several task-group meetings were organized in the framework of the annual assembly)

EULEP Workshop on "Comparative pathology of in vivo and in vitro systems"
Organised by the EULEP Committee of Pathology

Reisensburg (D), 10 March 1987
54 participants from 9 countries and the Commission

Principal subjects:
- Experimental induction of bone tumours in vitro and in vivo
- Search for oncogenes involved in the induction of osteosarcoma
- In vitro and in vivo studies on liver cells
- Transplants of human colon and lung cancer into nude mice
- Transplantable rat lung tumours studied in vitro and in vivo

EULEP Workshop on "Macrophages"
Organised by the EULEP Committee of Internal Radiation Dosimetry and Techniques

Reisensburg (D), 12 March 1987
58 participants from 9 countries and the Commission

Principal subjects:
- Classification and pathophysiology of pulmonary macrophages
- Immunological properties of macrophages
- Dissolution of particles in macrophages; studies on lysosomes
- Response of pulmonary macrophage populations to irradiation
- Local migration of alveolar macrophages during particle clearance
- Macrophages in bone

EULEP Task Group Meeting on "Beta-irradiation of mouse and pig skin"

Berkeley (GB), 27 April 1987
8 participants from 3 institutes (1 country)

Principal subjects :
- Irradiation of mouse and pig skin with small sources of ^{90}Sr and ^{170}Tm
- Acute damage to the dermal layer
- Regenerative response

International Seminar : "Foodstuff intervention levels following a nuclear accident"

Luxembourg (L), 27-30 April 1987
86 participants by invitation from 25 countries and 5 international organizations, and the Commission

Principal subjects:
- Intervention levels of dose
- Models applicable to the calculation
- Important parameters in the models and appropriate values for these parameters
- International and national approaches adopted subsequent to the Chernobyl reactor accident
- Contaminated animal feedingstuffs and fertilizer

EULEP Training Course on "Fundamentals of Molecular Biology", a practical course organised by the EULEP Committee of Cell and Molecular Biology

Mol (B), 5-15 May 1987
12 course members from 6 countries and the Commission

Principal subjects:
- isolation of RNA and DNA from mammalian tissues
- gel electrophoresis of RNA and DNA samples
- use of restriction enzymes, mapping of DNA
- hybridization of immobilized RNA or DNA
- synthesis of a radio-labelled molecular probe

Workshop on "Low dose radiation and the immune system"
Co-organized with the Kernforschungsanlage Jülich (D), Electric Power Research Institute, Palo Alto (USA) and the US-Department of Energy, Washington DC

Dreieich (D), 7-8 May 1987
35 participants from 7 countries and the Commission

Principal subjects:
- Low dose effects on the cellular level
- Immunology of radiation induced cancer
- Effects of radiation on immune cells
- Effects of radiation on immune functions
- Observations in man

Workshop on "Development of personal neutron dosemeters based on track etch detectors"
Co-organized with Eurados (European Radiation Dosimetry Group) and the UKAEA (UK Atomic Energy Authority), Harwell (GB)

Harwell (GB), 12-14 May 1987
47 participants from 12 countries

Principal subject:
- Research and progress in individual neutron dosimetry using track etch detectors

EULEP Task Group Meeting on "Late vascular changes in irradiated brain"

Rotterdam (NL), 15 May 1987
6 participants from 4 countries

Principal subjects:
- multi-disciplinary approach to radiation effects on the adult brain
- late appearance of decreased vascular density and vasodilatation
- blood vessel permeability changes
- development of abnormal blood vessels

"Fifth information seminar on the radiation protection dosemeter intercomparison programme"
Co-organized with ENEA, Bologna

Bologna (I), 25-27 May 1987
49 participants from 11 countries and the Commission

Principal subjects:
- Presentation of the results of the 1986 intercomparison programme for personal beta dosemeters
- Discussion and comments on the results
- Various contributions on beta dosimetry
- Miscellaneous topics relating to radiation protection dosimetry

Workshop on "Consequences of an accidental contamination of the urban environment"
Co-organized with the Risø National Laboratory, Roskilde

Roskilde (DK), 9-12 June 1987
100 participants from 18 countries and the Commission

Principal subjects:
- Deposition and run-off
- Weathering and resuspension
- Shielding factors and indoor dose evaluation
- Reclamation and relocation
- Countermeasures and socio-economic consequences

International Colloquium on "Radioactivity and oceanography"
Co-organized with the Institut National des Techniques de la Mer, Cherbourg

Cherbourg (F), 1-6 July 1987
150 participants from 14 countries and the Commission

Principal subjects:
- Sources and applications of radionuclides in the marine environment
- Study of large scale oceanographic processes by means of natural radionuclides
- Natural radionuclides used as tracers of scavenging process in the ocean and in coastal waters
- Natural radionuclides and sedimentary marine processes
- Fallout from Chernobyl : an oceanographic tracer
- Use of radionuclides and processes of coastal and estuarine sedimentation
- Development and application of models in connection with radionuclides in the marine environment

Seminar "Information about radiation protection" for the representatives of the European Trade Union Confederation

Luxembourg (L), 8-9 July 1987
20 participants from 12 countries and the Commission

Principal subject:
- Information about the main activities of the Commission in the field of radiation protection

Workshop on "Low dose radiation effectiveness and mechanisms of high-LET particles
Co-organized with the US-Department of Energy, Washington DC

Darmstadt (D), 16-17 July 1987
30 participants from 7 countries and the Commission

Principal subjects:
- Discussion of 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

Workshop on "Environmental dose-ratemeters"
Co-organized with the PTB Braunschweig and the Risø National Laboratory, Roskilde

Braunschweig (D) and Roskilde (DK), 10-14 August 1987
15 participants from 6 countries and the Commission

Principal subjects:
- Establishment of practical methods for environmental gamma monitoring instruments
- Intercomparison of instruments in different experiments (free field measurements of the natural background radiation, measurement of cosmic ray component at sea, determination of the inherent instrumental background, calibration with the various radioactive sources

EULEP Workshop on "Endocrine tumours and the APUD system"
Organised by the EULEP Committee of Pathology

Uppsala (S), 25-26 September 1987
21 participants from 8 countries

Principal subjects:
- Development of the Amine Precursor Uptake and Decarboxylation system
- Differentiation markers
- Proliferative lesions involving the APUD system
- Immunohistochemical studies of normal tissue and tumours
- In situ hybridization for neuroendocrine tumours

EULEP Workshop on "Stem cells in bone marrow after contamination with osteotropic radionuclides"
Organised by a Task Group of EULEP

Antwerp (B), 29-30 September 1987
27 participants from 7 countries

Principal subjects:
- Short- and long-term effects on pluripotent stem cells
- Spatial distribution of stem cells within bone marrow
- Studies using ^{224}Ra, ^{226}Ra, ^{239}Pu, ^{241}Am
- Bone progenitor cells in the bone marrow
- Effects on the microenvironment of stem cells

EULEP Task Group Meeting on "Bone-seeking radionuclides"

Antwerp (B), 30 September 1987
12 participants from 4 countries

Principal subjects:
- Pu and Am distribution in baboon bones
- ^{226}Ra distribution in beagle dog and pig bone
- distribution of different actinides in mouse skeleton
- long-term retention and toxicity of ^{237}Np in rats
- lymphoma induction in the mouse from repeated ^{224}Ra injections

Standing Conference "Health and safety in the nuclear age"

Luxembourg (L), 5-7 October 1987
120 participants from 13 countries and the Commission

Principal subject:
- Information of the public and the media on healthy protection and safety with regard to nuclear activities

Workshop on "Methods for assessing the reliability of environmental tranfer models predictions".
Co-organized with the Greek Atomic Energy Commission, Athens, the US - Department of Energy, Washington, DC, and the International Atomic Energy Agency, Vienna.

Athens (GR), 5-9 October 1987
98 participants from 21 countries and the Commission

Principal subjects:
- Overview of model reliability and testing
- Tranfer air-land
- Transfer in the terrestrial environment
- Transfer in the aquatic environment
- Transfer in the biosphere from waste repositories
- Uncertainty analysis

EULEP Task Group Meeting on "Inter-species comparison of lung clearance"

Cadarache (F), 6-7 October 1987
8 participants from 4 countries

Principal subjects:
- interspecies comparison of lung clearance of cobalt oxide particles
- studies with cobalt oxide particles of different solubilities
- related aspects of cobalt retention in the respiratory tract
- measurements of intra-lysosomal pH in cultured alveolar macrophages
- dissolution rates of particles in cultured alveolar macrophages

Sixth Symposium of Neutron Dosimetry
Co-organized with the GSF (Gesellschaft für Strahlen- und Umweltforschung), Neuherberg (D), and co-sponsored by the US-Department of Energy, Washington DC and ECNEU (European Clinical Neutron Dosimetry Group)

Neuherberg (D), 12-16 October 1987
203 participants from 21 countries and the Commission

Principal subjects:
- State-of-the-art of neutron dosimetry in radiation protection
- Therapy with particular focus on the implications of the possible change of the quality factor Q

Seminar on "Radiation protection in nuclear power plants"

Luxembourg (L), 19-20 October 1987
24 participants from 7 countries and the Commission

Principal subjects:
- Analysis of data provided by the 1986 questionnaires on job related doses
- Exchange of experience and information on radiation protection in nuclear power plants

EULEP Task Group Meeting on "Treatment of incorporated actinides"

Mol (B), 12 November 1987
12 participants from 4 countries and the Commission

Principal subjects:
- new preparation of decorporating agent LICAM(C)
- enhanced removal of plutonium from animals by injection of LICAM(C)
- toxicology of LICAM(C)
- proposed collaborative study using the new agent DFO-HOPO

EULEP Meeting for new Task Group on "Fetal dosimetry and effects of incorporated radionuclides"

Mol (B) 13 November 1987
20 participants from 6 countries and the Commission

Principal subjects:
- radionuclide distribution in the fetus and newborn
- assessment of dose to fetal tissues
- effects on the developing organism
- tissue sensitivity changes during gestation
- development of dosimetric models for man

EULEP Task Group Meeting on "Molecular approach to radiation-induced osteosarcomas and lymphomas"

Munich (D), 19-20 November 1987
19 participants from 4 countries

Principal subjects:
- retroviral sequences in radiation-induced lymphomas
- structure and function of murine leukaemia viruses
- RNA from cellular genes in virus-induced lymphoma?
- tests for direct radiation-induction of lymphoma by retrovirus
- comparative analysis of viruses causing osteomas and lymphoma

Symposium on "Natural Radiation Environment IV"
Co-organized with the LNETI, Sacavem and the US - Department of Energy, Washington, DC

Lisboa (P), 7-11 December 1987
220 participants from 22 countries and the Commission

Principal subjects:
- Natural radionuclides in the ground
- Natural radionuclides in the outdoor atmosphere
- Natural radionuclides in ecosystems
- Radon sources, migration and ingress into dwellings
- Radon decay in the indoor atmosphere
- Radon metrology
- Radon and radiation measurements and surveys
- Technologically enhanced levels of natural radioactivity
- Dose and risk assessment
- Radon reduction measures and control

C

MEETINGS OF EXPERTS IN 1987

Joint meeting of Article 37 experts and Member States'representatives

Luxembourg (L), 10-11 February 1987
54 participants from 12 Member States and the Commission

Principal subject:
- Nuclear power station discharge limits

Group of Experts referred to in Article 37 of the Euratom Treaty

Luxembourg (L), 12 March 1987
40 participants from 10 Member States and the Commission

Principal subject:
- Belleville (F) nuclear power station

Group of Experts referred to in Article 31 of the Euratom Treaty

Luxembourg (L), 5 May 1987
29 participants from 12 Member States and the Commission

Principal subjects:
- Euratom Basic Safety Standards
- Radiological consequences of the nuclear reactor accident at Chernobyl

Group of Experts referred to in Article 37 of the Euratom Treaty

Luxembourg (L), 2-3 June 1987
40 participants from 10 Member States and the Commission

Principal subject:
- Nogent-sur-Seine (F) nuclear power station

Group of Experts referred to in Article 31 of the Euratom Treaty

Luxembourg (L), 15 July 1987
29 participants from 12 Member States and the Commission

Principal subjects:
- Euratom Basic Safety Standards
- Radiological consequences of the nuclear reactor accident at Chernobyl

Meeting of Member States'representatives

Luxembourg (L), 21-23 October 1987
27 participants from 8 Member States and the Commission

Principal subject :
- The disposal of radioactive wastes into the Northeast Atlantic, their dispersion and the consequent exposure of the population (the MARINA project)

Meeting of Member States'representatives

Luxembourg (L), 26-27 October 1987
27 participants from 8 Member States and the Commission

Principal subject :
- The content, format comparability to the Commission under the terms of articles 35 and 36 of the Euratom Treaty

Group of Experts referred to in Article 37 of the Euratom Treaty

Luxembourg (L), 9-10 November 1987
40 participants from 10 Member States and the Commission

Principal subject:
- Isar (D) and Emsland (D) nuclear power stations

Group of Experts referred to in Article 31 of the Euratom Treaty

Brussels (B), 3 December 1987
29 participants from 12 Member States and the Commission

Principal subject :
- Euratom Basic Safety Standards
- Radiological consequences of the nuclear reactor accident at Chernobyl

V

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SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE

OF THE COMMISSION

CHOIX DE PUBLICATIONS FDITEES A L'INITIATIVE

DE LA COMMISSION

V. Publications 1987

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 1987, are given on the following pages.

A Preliminary Assessment of the Radiological Impact of the Chernobyl Reactor Accident on the Population of the European Community

M. MOREY et al., NRPB (UK), prepared under contract to the Commission.

The aim of the study was to review the environmental contamination measured in Member States of the European Community; to make a preliminary assessment of individual and population doses for each country; to make an estimate of the resulting health impact and to indicate the effects of the various preventive measures instigated by Member States in terms of the reductions in both individual and population exposure which they produced.

EUR Report 11523 EN 1987, 44 pages

Available on request to

DG/XI/A
CEC
L-2920 Luxembourg

The transfer of radionuclides through foodchains following accidental releases to atmosphere

Edited by J.R. SIMMONDS, C. STEINHAUER and S.M. HAYWOOD.

In the event of an accidental release of radionuclides to atmosphere countermeasures, such as the imposition of restrictions on food supply distribution, would be introduced to prevent serious radiation exposure due to the intake of foodstuffs. There would, nevertheless, be some exposure of the population due to the ingestion of foodstuffs contamined at low levels. In addition, the introduction of countermeasures carries a social and economic penalty which has to be included in any complete assessment of the consequences of accidental releases of radioactive materials to the environment. Assessments of the transfer of radionuclides through terrestrial foodchains are therefore carried out in accident consequence assessments to determine the extent to which countermeasures will affect agricultural production, and the health effects that would result from eating food products not affected by countermeasures.

The transfer of radionuclides through foodchains is being considered as one part of a study of methods for assessing the radiological impact of accidental releases of radionuclides. The aim of this part of the study is to make recommendations on the most appropriate type of foodchain model and to identify the limitations of various modelling systems. This report contains a review of the existing terrestrial foodchain models for use in accident assessments. Various investigations relating to the transfer of radionuclides through terrestrial foodchains were carried out as part of this project. These have been reported in detail elsewhere, but the relevant results and conclusions are also summarised in this report.

The majority of this report is concerned with the transfer of radionuclides to terrestrial foodchains as this is an important long term exposure pathway following accidental releases to atmosphere.

EUR Report 11255 EN, 1987, 35 pages, 5 figures, 4 tables.

To be ordered through :

Office for Official Publications
of the European Communities
Boîte postale 1003
L-2985 Luxembourg

Price : ECU 4.60, BFR 200

Occupational radiation dose statistics from light water power reactors operating in Western Europe

Final report of contract between the Commission of the European Communities, the Central Electricity Generating Board and the Swedish State Power Board

Edited by I.R. BROOKES and T.ENG

Since the early days of nuclear power, collective and individual doses for people engaged in the maintenance and operation of nuclear power plants have been published by regulatory authorities.

In 1979 a small working party whose members were drawn from Member States operating light-water reactors (LWRs) in the European Community was convened.

The working party decided that only by collection of data under a unified scheme would it ever be possible to properly compare plant performance and for this reason a questionnaire was drawn up which attempted to elicit the maximum of information with the minimum inconvenience to the plant staff. Another decision made by the working party was to broaden the data base from "European Community LWRs' to "West European LWRs' to try to take advantage of the considerable experience being built up in Sweden, in Finland and in Switzerland.

All the data available to the Commission up to the end of 1984 are presented and commented on. The deductions are not exhaustive but are believed to represent the limits of what could sensibly be done with the data available.

Results are presented separately for BWRs and PWRs but no other subdivision, say by country or maker, is made. Where interpretation can be enhanced by graphical presentation, this is done. In general, doses for each job category are expressed in various ways to reveal and afford comparisons.

Radiation protection series ; N° 36
EUR Report 10971 EN, 1987, 228 pp

To be ordered through :

Office for Official Publications
of the European Communities
Boîte postale 1003
L - 2985 Luxembourg

Price (excluding VAT) in Luxembourg
ECU 16,40 BFR 700 USD 18.90

Exposure to Natural Radiation in Dwellings in the European Communities

Report adopted by the Group of Experts appointed under the terms of Article 31 of the Euratom Treaty.
Report prepared by J.P. McLAUGHLIN, Univ. Coll. Dublin, under contract to the Commission

The Report considers the nature of radon exposure in dwellings, methods of measurement, estimated doses and eposemiological studies as well as reviewing the results of national and regional surveys in Member States and in other countries.
Finally, it considers current national regulatory approaches and relevant recommendations of various international organizations.

Doc. N° V/6683/87, May 1987, 115 pp
Available on request from:

DG/XI/A
CEC
L-2920 Luxembourg

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.

EUR Report 11449 EN, 1988, 283 pages, in press

To be published by:

Office for Official Publications
of the European Communities
L-2985 Luxembourg

Real-time Computing of the Environmental Consequences of an Accidental Release to Atmosphere from a Nuclear Installation

Proceedings of a Workshop organized by the Commission,
Luxembourg (L), 17-20 September 1985

The Workshop contained presentations of current models (including demonstrations); aspects of the modelling process; the provision of input data; uncertainties and validation considerations; the user's requirements and the cost-effectiveness of models in relation to their sophistication, speed and accuracy.

Doc. N° V/2943/86, November 1987, 700 pp
Available on request:

DG XI/A
CEC
L-2920 Luxembourg

Radiological mass-screening within the Member States of the European Communities. Regulations, Practices, Effectiveness

Proceedings of the Seminar jointly organized by the Commission of the European Communities , the Commissariat à l'Energie Atomique, CEA and the Centre d'études sur l'évaluation de la protection dans le domaine nucléaire, CEPN (France).
Luxembourg, 3 - 4 December 1985

Edited by Jacques LOCHARD (CEPN)

A technical workshop on practices and regulations in the field of radiological mass-screening within the Member States (Luxembourg, 4 - 5 December 1984) and a seminar on the same subjects (Luxembourg, 3 - 4 December 1985) were organised with a view to collecting information on effectiveness and cost of radiological mass-screening, undertaken in the Member States. The seminar provided the representatives of the national authorities responsible for radiation protection, public health and occupational medicine with an opportunity to discuss mass-screening practices with experts.

The proceedings contain the papers presented at the workshop and the seminar. It gives an overview on regulations, current practices and effectiveness of radiological mass-screening and it is hoped that it will help the responsible authorities in the Public Health sector in their planning activities and decision making.

Radiation Protection series - 37
EUR Report 11059, 1987, 494 pp

To be ordered through

Office for Official Publications
of the European Communities
L-2985 Luxembourg

Price (excluding VAT) in Luxembourg:

ECU 37.20, BFR 1,600, US$ 42.50

Age-related factors in radionuclide metabolism and dosimetry

Proceedings of a Workshop jointly organized by the Commission of the European Communities and the CEA-IPSN (Commissariat à l'Energie Atomique - Institut de Protection et de Sûreté Nucléaire), Fontenay-aux-Roses (F) Angers (F), 26-28 November 1986

Edited by G.B. GERBER, H. METIVIER and H. SMITH

The proceedings present a review of data and models for radionuclide metabolism and dosimetry usable for the general public and especially for foetus, infant and child. While ICRP has made recommendations for such models for workers it has not done so for the general public; the critical population may include embryos, foeti, infants and children, age-groups whose radionuclide metabolism and organ dosimetry differ markedly from that of adults. These aspects have become particularly timely after the Chernobyl accident.

The forty-five contributions cover the following aspects of age-dependent radionuclide metabolism:

- gastrointestinal uptake,
- inhalation pathways of radionuclides in infants and children,
- metabolism of radionuclides in developing bone, thyroid and other organs,
- transfer of radionuclides to the foetus,
- models for metabolism and dosimetry of radionuclides in foeti, infants and children.

A roundtable discussion reviewed the needs for further research and the possible implications for appropriate recommendations in radiation protection.

EUR Report 10556 EN, published by Martinus Nijhoff Publishers, 1987, 416 pages

To be ordered through:

Kluwer Acadamic Publishers Group
Distribution Centre
P.O. Box 322
NL-3300 AH Dordrecht

Price: HFL 230

Human Exposure to Ultraviolet Radiation: Risks and Regulations

Proccedings of a Seminar organized by the Dutch Ministries responsible for Public Health and for the Environment and co-sponsored by the Commission of the European Communities.
Amsterdam, 23-25 March 1987.

Edited by W.F. PASSCHIER and B.F.M. BOSNJAKOVIC

The effects of ultraviolet radiation on human health were discussed. The seminar also considered the risks associated with actual UV exposures, studied in what way people may protect themselves and firally reflected about the regulations proposed or in existence in different countries.

The proceedings contain 75 contributions including 11 invited papers and a recording of the plenary discussions. The conclusions of the seminar indicated that the normal use of sunbeds, especially those with pure UVA light, should not give rise to particular concern, that the regulation of UV in the workplace is not difficult, but the regulation of the exposure from sun holidays and sunbeds is not needed although education and information for the public about the sensible attitude to UV exposure is becoming increasingly desirable.

Excerpta Medica (International Congress Series 744) published by Elsevier Science Publishers BV (Biomedical Division), 1987, 580 pages

To be ordered through:

Elsevier Science Publishers BV (Biomedical Division)
PO Box 211
1000 AE Amsterdam
The Netherlands

Price: ECU 150
H.Fl. 350

Foodstuffs intervention levels following a nuclear accident

Proceedings of the International Scientific Seminar organized by the Commission of the European Communities
Luxembourg (L), 27-30 April 1987

The Proceedings summarise the scientific discussions on the methodologies applicable to calculating derived intervention levels for foodstuffs for human consumption following accidental contamination of the environment. The following topics are covered : intervention levels of dose, models applicable to the calculation, important parameters used in the models and appropriate values for these parameters, international and national approaches adopted subsequent to the Chernobyl reactor accident, and contaminated animal feedingstuffs and fertilizers.
The roundtable discussion is presented, during which the chairmen reviewed the presentations and where a poll was taken as to participants opinions on the levels applicable to caesium in milk and meat.

EUR Report 11232 EN, 1987, 450 pp
Available on request

DG XI/A
CEC
L-2920 Luxembourg

Low dose radiation and the immune system

Proceedings of a Workshop jointly organized by Electric Power Research Institute, US Department of Energy, Commission of the European Communities and Jülich Nuclear Research Centre.
Dreieich - Frankfurt (D), 5-8 May 1987

Scientists from Europe, the USA and Japan discussed the possible effects of low radiation doses on the immune system. 19 papers dealt with microdosimetric and molecular aspects, the cellular and molecular mechanisms of tumour initiation and tumour growth by low doses, the response of the immune system components to low doses, and the comparison of low level radiation suppression of the immune system with that associated with ageing. A round table discussion analysed possible mechanisms of action of radiation on the immune system and their consequences and developed ideas for future research needs.

EUR Report 11140 EN
Published in International Journal of Radiation Biology, Vol. 53, No. 1, January 1988, 204 pages.

To be ordered through:

Taylor and Francis Ltd
Rankine Road
Basingstoke
GB-Hants RG24 OPR

Price: £ 30

Etched track neutron dosimetry

Proceedings of a Workshop jointly organized by the Commission of the European Communities and the UKAEA (UK Atomic Energy Authority), Harwell and Eurados (European Radiation Dosimetry Group)
Harwell, 12-14 May 1987

Edited by: D.T. BARTLETT, J. ECOZ, K.G. HARRISON

The current proposals to increase the neutron quality factor for radiation protection requires improvement of the sensitivity and response of individual dosemeter systems. The etched track detector CS-39 which is the material commonly used for plastic spectacle lenses looks very promising in this respect.

About 50 scientists from fourteen countries discussed the state of development of these detectors. Proton-sensitive etched-track detectors offer higher sensitivity and greater accuracy than the nuclear emulsion which has been the principal personal neutron dosemeter for over 30 years. Further increase in sensitivity and improvement of response for low energy neutrons can be achieved by improving the homogeneity and reliability of the CR-39 plastic.

Eight invited review papers and sixteen contributed papers were presented and discussed at the workshop. The proceedings include also a summary of the discussion.

EUR Report 11242 EN, published in Radiation Protection Dosimetry, Vol. 20, No.s 1/2, 1987, 130 pages

To be ordered through:

Subscription Department
Nuclear Technology Publishing
P.O. Box N° 7
GB - Ashford, Kent

Price: £ 30

Beta Dosimetry - Fifth Information Seminar on the radiation protection dosemeter intercomparison 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

EUR Report 11363 EN, 1988, about 300 pp, in press

To be published by:

Office for Official Publications
of European Communities
L-2985 Luxembourg

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.

EUR Report 11235, 1988, in press.

To be published by :

Radiation Protection Dosimetry
Nuclear Technology Publishing
P.O. Box 7
Ashford
GB-Kent TN25 4NW

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 2.8 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 1988.

For information :

C.E.C.
DG XII/D
Rue de la Loi, 200
B - 1049 Brussels

VI

VERZEICHNIS DER FORSCHUNGSGRUPPENLEITER

LIST OF RESEARCH GROUP LEADERS

INDEX DES CHEFS DE GROUPE DE RECHERCHE

RESEARCH GROUP LEADERS

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

Europäische Gemeinschaften — Kommission
European Communities — Commission
Communautés européennes — Commission

EUR 11464 - Tätigkeitsbericht — Programm Strahlenschutz — 1987
Progress report — Radiation protection programme — 1987
Rapport d'activité — Programme Radioprotection — 1987

Luxembourg: Office for Official Publications of the European Communities

1988 – X, 1 753 pp. – 16,2 × 22,9 cm

Radiation protection series

DE/EN/FR

ISBN 92-825-8337-6

Kat./cat. : CD-NA-11464-3A-C

Preis in Luxemburg (ohne MwSt.) :
Price (excluding VAT) in Luxembourg : ECU 81
Prix au Luxembourg, TVA exclue :

The progress report of the radiation protection programme outlines the research work carried out in the year 1987 under contracts between the Commission of the European Communities and research groups in the Member States. Results of more than 320 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.

Navn

Institut

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Fordelingskoden er tilpasset strålingsbeskyttelsesprogrammets forskellige arbejdsområder. De rubrikker, der svarer til Deres interessefelter, bedes forsynet med et X.

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- ☐ 3. Strålingsvirkninger på kort sigt, akut strålingssyndrom og dets behandling.
- ☐ 4. Strålingsvirkninger på lang sigt og inkorporerede radionukleiders toksikologi.
- ☐ 5. Strålingsmåling og dens fortolkning; dosimetri.
- ☐ 6. Vurdering af strålingsrisici.

Såfremt De er interesseret i at blive optaget på vor forsendelsesliste, bedes De tilbagesende dette kort i udfyldt stand (maskinskrevet).

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